The Journal of Laboratory and Clinical Medicine

VOL. 23 AUGUST, 1938 NO. 11

CLINICAL AND EXPERIMENTAL

CONGENITAL CYSTIC LUNG DISEASE

DEAN B. COLE, M.D., AND WALTER L. NALLS, M.D., RICHMOND, VA.

FONTANOUS is credited with the first description of congenital cystic lung disease. In 1638 he reported the case of a three months old infant dying in an attack of cyanosis who had a large bladder of air which communicated with the bronchi. There were six additional cases reported up to 1687, when Bartholinus reported a case of cystic disease of the upper lobe of the left lung in a four-year-old child. Until 1925 congenital cystic lung disease was considered a rare condition. In that year Koontz was able to collect 108 cases from the world's literature and added a case of his own. He states that most of these cases had been reported by the Germans, a few by the French, Italians, and English, and that no American cases were found in the literature. Oechsli and Miles state that the first American case was reported by Pappenheimer in 1913, and that no additional cases were described in this country until that of Koontz. The report of Koontz did much to stimulate interest in the condition in this country and since the appearance of his paper, reports of cases have appeared in the literature with increasing frequency. In 1936 Schenck was able to collect 124 additional cases reported throughout the world, more than half of which were by Americans. Again in July, 1937, this same author collected an additional 140 cases which had appeared since his previous report, making a total of 381 cases up to that time.

The condition has been designated by such terms as congenital bronchiectasis, fetal bronchiectasis, bronchiolectasis, telangiectatica, honeycombed lung, congenital cystic malformation of the lung, cystic fetal bronchial adenoma, and

Received for publication, January 3, 1938.

congenital pulmonary lymphangiectasis by different authors in an attempt to describe the underlying pathogenesis. The term congenital cystic lung disease has been generally adopted to include all of these bizarre types of the same condition which is now becoming more frequently recognized, clinically as well as at autopsy.

There is no unanimity of opinion regarding the etiologic factor in congenital cystic lung disease. This accepted designation implies that the etiologic basis, if not the lung cyst itself, had its origin before birth. Although this view is held by most writers on the subject, there are some who believe the condition may occur as the result of postnatal mechanical or infectious influences. The role of infection in the causation of the disease has been generally disproved. Many cases have never shown evidence of infection and in others in which infection had occurred it has been shown to have been superimposed upon the primary abnormality. Admitting that pulmonary cystic disease is due to a developmental error, there are many factors in the genesis of the condition that are unknown and about which there is disagreement.

The cyst walls may have the characteristics of bronchi, bronchioles, atria, infundibula, or alveoli. The cyst cavity is often traversed by strands of fibrous tissue which may give the cyst the appearance of being trabeculated or multilocular. They usually contain a varying amount of gelatinous or albuminous fluid with desquamated epithelial cells and debris. If infected, pus will be present. The wall of the cyst may be thin or thick, depending upon the size of the cavity, the pressure therein, and the presence or absence of infection. In many instances the quantity of fluid is not sufficient to be demonstrable on x-ray and in such cases the cyst appears only to contain air. In other cases the cyst may be completely filled with fluid and have the appearance of a solid tumor of the lung. The cyst may be only a few millimeters in diameter, or may be so large as to replace an entire lobe or lung, and even displace the mediastinum and encroach upon the contralateral lung. They may be single or multiple and involve one or all lobes of the lung. In the large balloon type of cyst, the walls are usually thin and are easily ruptured. The small cysts as a rule have thicker walls and seldom perforate. Bronchial communication is important because of its relationship to infection. When such communication exists, infection of the cyst is quite common. On the other hand, when no such communication is present, infection rarely occurs. If the bronchial communication remains patent, the pressure relationships in the cyst are not changed, but often a ball-valve mechanism is produced in the bronchial communication allowing air to become entrapped in the cyst, causing a progressive ballooning with accompanying pressure symptoms.

The disease may be present for years without producing symptoms and be discovered in routine x-ray examination or incidentally at autopsy when death has been due to some other cause. When symptoms are present they are dependent upon the size, location, number, and contents of the cysts, the nature of the communication with the bronchi, if any, the presence or absence of infection, and the possibility of rupture into the pleural cavity producing pneumo-

thorax. In infants and young children the occurrence of repeated sudden attacks of dyspnea and cyanosis is suggestive of cystic disease with or without possible tension pneumothorax. Among the more frequent localizing symptoms are cough, dyspnea, cyanosis, hemoptysis, and less frequently purulent sputum and chest pain. Infected cysts may simulate bronchiectasis, lung abscess, tuberculosis or localized empyema.

The physical examination is usually of little value, since small multiple cysts may show no characteristic findings and the larger cysts are often confused with localized emphysema or pneumothorax. Accurate diagnosis during life is dependent largely upon the proper interpretation of x-ray films. Bronchoscopy may show abnormalities of the bronchi or may not be helpful. Lung mapping with iodized oil is diagnostic in some cases, but without value in others. In the type of the disease with a single large cyst, pneumothorax may be established, thus outlining the cyst wall.

Relatively few cases are suitable for and respond to any specific form of treatment. The injection of various chemicals into the cyst has resulted in a few cases in its obliteration. Surgical removal of the cyst, lobectomy, and pneumonectomy have each been successful in a limited number of cases. Pressure symptoms can often be relieved by the aspiration of air from the cyst, or from the pleural cavity if pneumothorax is present. Finally, it must be remembered that treatment will be dependent upon the size, number, location, and type of cyst, the presence or absence of infection, and the urgency for relief of symptoms.

CASE REPORTS

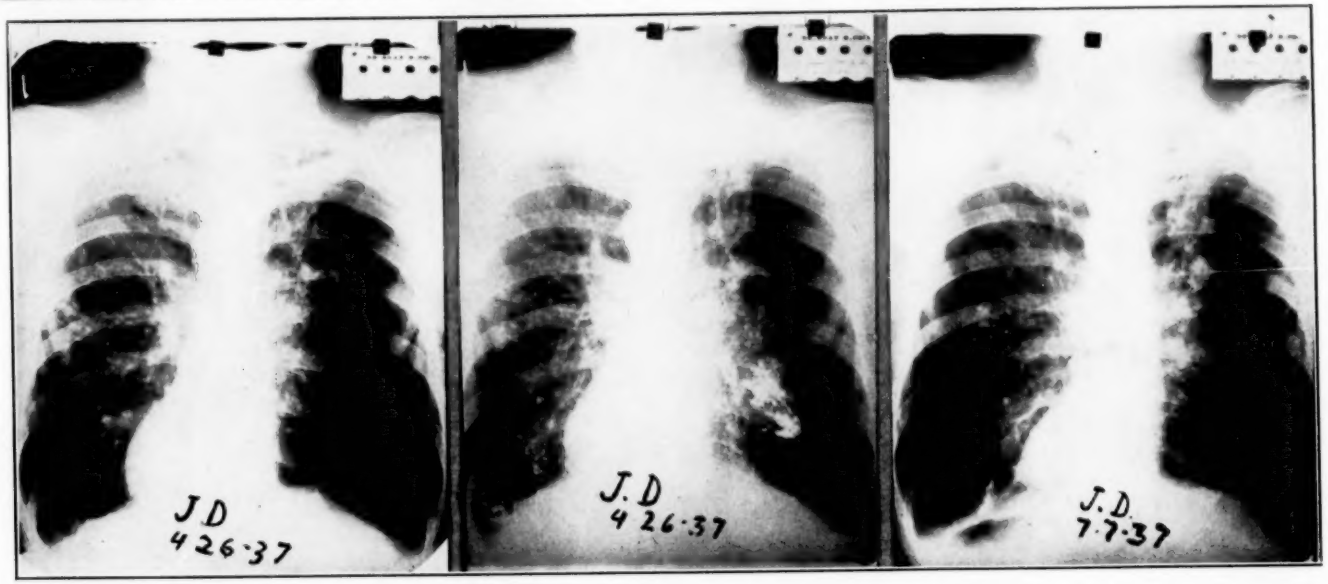
CASE 1.—A white male, 59 years of age, was first seen April 21, 1937, because of a recurring, paroxysmal, productive cough with blood-streaked expectoration. Although these symptoms had been present for thirty years, only recently he had developed marked weakness, fatigue, and dyspnea.

During examination he coughed constantly and expectorated freely and was moderately dyspneic. There was pronounced bronchovesicular breathing, with a few small moist and bronchial râles over the upper lobes and diminished breath sounds with tympany over the bases. Other physical findings and laboratory studies were not significant.

Stereoscopic roentgenograms of the chest showed a bilateral upper lung tuberculosis which had healed by fibrosis and calcification. At both bases there were large trabeculated areas of lessened density which were interpreted as lung cysts.

A bilateral lung mapping was done, but no lipiodol entered the cystic areas. Lipiodol was then injected directly into the cyst in the right lung through the chest wall. Fluoroscopy and x-rays immediately thereafter showed a developing pneumothorax as the result of the needle puncture. The then partially collapsed lung cyst was well outlined by lipiodol. The cyst in the left lung was not punctured.

The patient returned to work and continued to improve without further treatment for two and a half months, when his cough suddenly became worse and he developed a progressive severe dyspnea. Roentgenograms of the chest at this time showed the cyst in the right lung to be much smaller in size. On the left there was a tension pneumothorax collapsing the entire lung base. This had occurred spontaneously and explained the sudden recurrence of symptoms. On introduction of a needle into the left pleural space a positive pressure was encountered and after the removal of 900 c.c. of air, the pressure was reduced to about normal. This immediately relieved the patient's dyspnea. Following this he was admitted to a sanatorium for observation. He has since had three recurrences of spontaneous pneumothorax.



A. *B.* *C.*

Fig. 1—Case 1. J. H. D. *A* (4/26/37) Showing large trabeculated cystic areas in right lung and smaller ones in left. Also considerable fibrosis and calcification in both upper lobes. *B* (4/26/37) Film made after iodized oil had been introduced directly into the cyst at the base of the right lung through the chest wall. Shows oil outlining the partially collapsed cyst; also area of pneumothorax and the cyst wall. *C* (7/7/37) Pneumothorax on left was spontaneous. Residual of induced pneumothorax in right costophrenic angle.

Case 2.—A 31-year-old white male was first seen September 26, 1937, at the office because of severe coughing with profuse expectoration, wheezing, loss of weight, and vomiting. There was a history of asthma since infancy which was severe until he was 12 years of age. He was then relatively free of asthma for the next fifteen years. His asthmatic symptoms returned about three years ago. The attacks were usually preceded by chills and fever, and were accompanied by severe coughing and expectoration. They recently had increased in frequency and severity. During the past year he had lost fifty pounds in weight. The present attack began with chills, fever, nausea, and vomiting eleven weeks previously. The asthma was so severe that he had been orthopneic the greater part of the time. He had been hospitalized six weeks previously and a diagnosis of advanced tuberculosis with bilateral cavitation had been made, although sputum studies had been negative for tubercle bacilli.

Examination revealed a malnourished white male who appeared acutely ill. He was dyspneic coughed continuously, and expectorated profusely. There was a noticeable offensive, sweetish body odor. The blood pressure was 90/70 and the pulse 120. Otherwise the heart was normal. Chest was emphysematous, and the lungs showed numerous bronchial

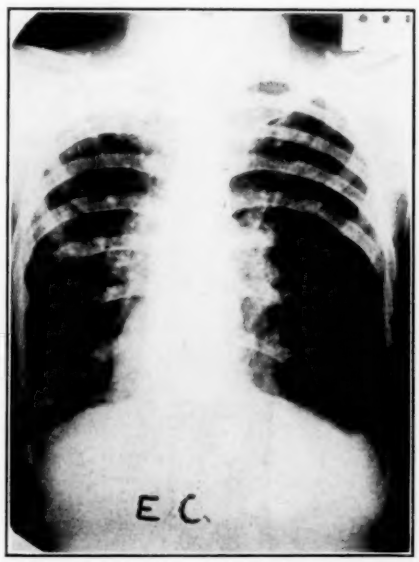


Fig. 2.—Case 2. E. C. Film showing an atypical fibrosis with multiple areas of lessened density due to cystic disease of the lungs. Diagnosis confirmed at autopsy.

and moist râles, with asthmatic breath sounds throughout. The abdomen was distended, and the liver and spleen were palpable. Lower extremities showed rather marked pitting edema.

Laboratory: urinalysis—amber, acid 1.015. Albumin: ++++ (boiled solid). Frequent pus cells. Few red blood cells. Hbg. 80 per cent, R.B.C. 4,200,000. R.B.C. showed marked stippling. W.B.C. 16,800, polys. 83 per cent, lymph. 17 per cent. Blood sugar 97.5 mg., N.P.N. 157.8 mg., creatinine 8.6 mg. Sedimentation index 24 mm. in fifteen minutes and 31 in an hour. Blood cultures negative. Blood Wassermann negative. Sputum examinations repeatedly negative for tubercle bacilli. Culture of sputum revealed a mixture of staphylococci and streptococci.

Electrocardiogram was normal.

X-ray of sinuses was negative.

The lungs on x-ray appeared emphysematous. There was an unusual mottling interspersed with multiple areas of honeycombing and many larger areas of lessened density, giving the appearance of cysts or blebs.

Patient was put to bed and despite treatment for his lung condition and nephritis, he became progressively worse and died October 2, 1937.

Autopsy: An emaciated young white male. There was some edema of the feet and ankles. Lungs appeared emphysematous. There were numerous bladderlike cysts throughout the lungs, varying in size from 0.5 to 4 cm. in diameter. They were more pronounced along the anterior border and in the upper lobes. Some of these contained air, others fluid, and others pus. Many of these cysts apparently communicated with the bronchi. There were numerous adhesions making it difficult to free the lungs. There was a basal bronchopneumonia in both lungs. Heart: There were a number of fine pericardial adhesions. The heart muscle was flabby and had a "faded leaf" appearance. There was a relative right-sided enlargement. The aorta was quite small. Thymus measured 3 by 2 by 2 cm. Abdomen: Liver was markedly enlarged and extended well into the pelvis and the cut surface appeared pale. The capsule was thickened. Spleen was enlarged and firm. Kidneys were large and pale. The capsule stripped easily. The cortex was swollen and the markings were obliterated. Stomach was small and of the "ram's horn" type.

Anatomical Diagnosis: (1) Congenital cystic lung disease with numerous infected cysts. (2) Persistent thymus. (3) Right-sided cardiac enlargement with a small aorta. (4) Acute nephritis. (5) Enlarged liver and spleen.

Summary: Congenital cystic lung disease is still a relatively rare condition, but is becoming recognized with much greater frequency as evidenced by the reports of more than 140 cases since 1936. This number is greater than had been reported in the world's literature prior to 1925. Most of the earlier reports were of cases occurring in infants and unrecognized until discovered at autopsy. The condition has since been found to occur at all ages, but despite this it is the consensus of opinion that it is a congenital abnormality. Because of the presence of infection in some cases, the condition may simulate tuberculosis or bronchiectasis. The condition is probably more common than generally believed and possibly is the pathologic basis for many cases of idiopathic spontaneous pneumothorax. In two of our cases repeated spontaneous pneumothoraces have occurred. One of these not recorded here is reported elsewhere because of a recurring simultaneous bilateral spontaneous pneumothorax.

Note: We wish to express our indebtedness to Dr. S. W. Budd of Richmond, Va., for the notes on the autopsy of the second case.

REFERENCES

Churchill, Edward D.: Lobectomy and Pneumonectomy in Bronchiectasis and Cystic Disease, J. Thoracic Surg. **6**: 286, 1937.

Freedman, Eugene: Congenital Cysts of the Lungs, Am. J. Roentgenol. **35**: 44, 1936.

Gale, J. W., Keeley, J. L., and Coon, H. M.: Total Pneumonectomy for Congenital Cystic Disease of the Lung, J. Thoracic Surg. **6**: 626, 1937.

Hasney, Fred A., and Baum, Felix: Bilateral Spontaneous Idiopathic Pneumothorax in Apparently Healthy Individuals, Radiology **28**: 47, 1937.

Koontz, Amos R.: Congenital Cysts of the Lung, Bull. Johns Hopkins Hosp. **37**: 340, 1925.

Oechsli, Waldo R., and Miles, Seth H.: Simultaneous Bilateral Spontaneous Pneumothorax, Am. Rev. Tubere. **30**: 239, 1934.

Oughterson, Ashley W., and Taffel, Max: Pulmonary Cysts, Yale J. Biol. & Med. **9**: 77, 1936.

Pollock, William C.: Pulmonary Cystic Disease, Am. Rev. Tubere. **34**: 527, 1936.

Schenck, Samuel George: Congenital Cystic Disease of the Lungs, Am. J. Roentgenol. **35**: 604, 1936.

Schenck, Samuel George: Diagnosis of Congenital Cystic Disease of the Lungs, Arch. Int. Med. **60**: 1, 1937.

Wood, Harry G.: Congenital Cystic Disease of the Lung, J. Thoracic Surg. **6**: 634, 1937.

NORMAL PLASMA PHOSPHATASE VALUES (JENNER-KAY METHOD)*

A. S. Mulay, Ph.D., and Samuel Hurwitz, M.D., San Francisco, Calif.

IN 1923 Robison[1] demonstrated an enzyme in the ossifying cartilage of young rats and rabbits capable of hydrolyzing phosphoric esters. Shortly thereafter the presence of phosphatase in the blood was described by Martland, Hansman, and Robison.[2] This enzyme has aroused increasing attention since its importance as a diagnostic and prognostic aid in pathologic conditions has become well established.[3, 4] However, there are only a few reports dealing with normal blood phosphatase values, and these have been obtained from a relatively small number of persons. In their work on phosphorus partition Stearns and Warweg[5] plot plasma phosphatase values against age. As this work is done by the Kay method, it is of little value for interpreting results obtained by the more commonly used Jenner-Kay method. Dr. Francis Scott Smyth suggested the need for more careful approximations of the phosphatase at various age levels. The purpose of this paper is, therefore, to present our determinations of plasma phosphatase on normal newborns, children, and adolescents convalescent from acute illness, and inmates of the San Francisco home for the aged.

There are three methods generally used for phosphatase determination, namely, the Jenner-Kay,[6] the Bodansky,[7] and the King method.[8] The fundamental basis for estimating phosphatase activity is the same in all methods. Since the enzyme has not been isolated in a pure state, the amount present in a given substance is calculated by measuring the phosphorus liberated as inorganic phosphate when the enzyme is allowed to act on a substrate under standard conditions. It is these latter factors that are variable in the different methods. We have utilized the Jenner-Kay Method in these determinations because it was the first technique described, and we do not believe that any other method yet described has sufficient advantages to replace it. Furthermore, any method is satisfactory for clinical application if the limitations are appreciated and normal standards are available.

DISCUSSION

The accompanying table shows the results of plasma phosphatase determinations by the Jenner-Kay method on persons who had no evidence of any abnormal condition known to alter phosphatase values. These figures are expressed in units. One Jenner-Kay unit represents that amount of enzyme which will liberate one milligram of phosphorus as free phosphate from an

*From the Department of Pediatrics and the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.

Aided by a grant from the Christine Breon Fund of the University of California.

Received for publication, January 18, 1938.

excess of disodium-B-glycerophosphate at pH 8.8 in three hours when kept at a temperature of 37.5° C. The phosphatase content of a sample of plasma is defined as the number of milligrams phosphorus which would be liberated by 100 c.c. of the plasma under the above conditions. Since the units of phosphatase activity adopted by different observers very considerably, it is difficult to compare their results. Brown,[9] however, has found that one Jenner-Kay unit equals approximately two Bodansky units. King[8] states that his unit is approximately equivalent to the Jenner-Kay unit.

TABLE I

PLASMA PHOSPHATASE VALUES

(Jenner-Kay Method)

AGE YEARS	NUMBER	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION 68.26 PER CENT	RANGE
Under 1 month	95	6.85	±0.14	±1.98	4.00-11.5
1 to 5	28	9.04	±0.28	±2.13	6.00-13.0
6 to 10	46	8.67	±0.21	±2.10	6.00-13.5
11 to 15	36	9.14	±0.31	±2.75	5.0 -14.0
16 to 20	25	5.98	±0.34	±2.44	4.0 -12.0
21 to 25	42	4.90	±0.21	±2.02	2.0 - 9.0
25 to 50	34	4.80	±0.15	±1.29	2.0 - 7.0
51 to 86	56	5.20	±0.15	±1.62	1.5 - 8.0

An analysis of the figures in Table I shows that in infants under one month of age the plasma phosphatase ranges between 4.0 and 11.5 units, averaging 6.85 units. Factors influencing this enzyme in the neonatal period are not clearly understood. The period of gestation, the condition of the mother, the number of ossification centers present at birth, are but a few conditions that need further investigation in relation to blood phosphatase values. From 1 to 15 years of age there is apparently an increase in plasma phosphatase, the values ranging from 5.0 to 14.0 units, with an approximate average of 9.0 units. This rise may possibly be associated with active growth at this period. We hope to report at a later date determinations of blood phosphatase on the same individuals at different ages to show the effect of growth on the enzyme activity. After 15 years of age there is rather rapid decrease in the phosphatase activity of the plasma until 25 years, after which period the values remain relatively the same throughout life. Beyond the age of 50 years, there is a wider fluctuation in phosphatase values with a lower minimal level (e.g., 1.5 units). The lower plasma phosphatase value with increasing age may possibly be explained by the decrease which occurs in the phosphatase activity of the bones partly by the larger proportion of inorganic matter in the older bones, but there may also be a reduction in the number and activity of the enzyme-secreting cells as postulated by Robison.[10]

SUMMARY

Normal plasma phosphatase values at different ages obtained by the Jenner-Kay method are detailed to serve as standards for clinical application.

REFERENCES

1. Robison, Y.: The Possible Significance of Hexosephosphoric Esters in Ossification, Biochem. J. **17**: 286, 1923.
2. Martland, M., Hansman, F. S., and Robison, R.: The Phosphoric-Esterase of Blood, Biochem. J. **18**: 1152, 1924.
3. Kay, H. D.: Phosphatase in Growth and Disease of Bone, Physiol. Rev. **12**: 384, 1932.
4. Bodansky, A., and Jaffe, H. L.: Phosphatase Studies. III. Serum Phosphatase in Diseases of the Bone; Interpretation and Significance, Arch. Int. Med. **54**: 88, 1934.
5. Stearns, G., and Warweg, E.: Studies of Phosphorus of Blood. I. The Partition of Phosphorus in Whole Blood and Serum, Serum Calcium and Plasma Phosphatase From Birth to Maturity, J. Biol. Chem. **102**: 749, 1933.
6. Jenner, H. D., and Kay, H. D.: Plasma Phosphatase. III. A Clinical Method for the Determination of Plasma Phosphatase, Brit. J. Exper. Path. **13**: 22, 1932.
7. Bodansky, A.: Phosphatase Studies. II. Determination of Serum Phosphatase Factors Influencing the Accuracy of the Determination, J. Biol. Chem. **101**: 93, 1933.
8. King, E. J., and Armstrong, A. R.: A Convenient Method for Determining Serum Bile Phosphatase Activity, Canad. M. A. J. **31**: 376, 1934.
9. Brown, P. C.: Blood Phosphatase. A Comparison of the Bodansky, Jenner and Kay, and King Methods, Am. J. M. Technol. **2**: 1, 1936.
10. Robison, R.: The Significance of Phosphoric Esters in Metabolism, New York, 1932, New York Univ. Press.

PIGEON RETICULOCYTES: AN UNCERTAIN INDEX OF HEMOPOIETIC ACTIVITY*

SAVAS NITTIS, M.D., DETROIT, MICH.

COINCIDENTAL with the great stimulus in the study of hemopoiesis, brought about by the discovery of the therapeutic value of liver and stomach in certain types of anemias, is the search for a suitable laboratory method for testing the potency of hemopoietic substances. Fortunately, from the beginning of this new hematologic era, an accurate clinical method already existed in the form of the so-called "reticulocyte reaction," introduced by Minot in 1926;[1] but a clinical method, even though accurate and reliable, has obvious serious limitations. In 1930, Vaughan and her co-workers[2] used the pigeon as an experimental animal for this purpose. Their work was repeated later by others[3] and, although their results showed serious numerical differences, they all claimed that liver extract had a hemopoietic effect in the grain-fed pigeon, which could be measured and expressed in terms of "reticulocyte response." All these groups of workers found that under certain standard conditions, the reticulocyte percentage in the peripheral blood of the pigeon is constant, and this percentage is increased after the administration of liver or liver extract. The "normal" level of the reticulocyte percentage varied between 10 and 40 per cent with the different groups of workers. Wills, in 1932,[4] demonstrated that large, spontaneous fluctuations interfere seriously with the constant level, supposedly obtained after keeping the pigeons on a diet of grain and water, and that such spontaneous reticulocyte reactions were, at times, greater than those obtained

*From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan, Ann Arbor.

Received for publication, December 31, 1937.

after feeding the birds with liver. Similar results were obtained also by Heimann and his co-workers.[5] Later Gurd[6] demonstrated that, with a suitable staining technique, the reticulocytes in the peripheral blood of the pigeon constituted about 99 per cent of all the circulating erythrocytes and, for this purpose, he advocated using only the heavily reticulated cells as indicators of erythropoietic reaction. Since, however, he obtained only negative results even with this procedure, he rejected the pigeon as a suitable animal for testing the hemopoietic activity of any substance and advocated instead the use of mammalian animals for such studies. However, despite these warnings, some workers continued using the pigeon as a test animal for hemopoietic activity, limiting counts to the heavily reticulated cells only.[7] That such obviously arbitrary distinction between heavily and nonheavily reticulated cells, introducing, as it does, an extremely hard to regulate personal factor in it, is unsuitable for any kind of serious study, requires no comment. The purpose of this paper is not to discredit the work of these investigators directly, but, by clarifying the mechanism of "vital staining," to explain the apparent discrepancies in their results and observations.

Experimenting with the vital staining of nucleated erythrocytes of various animals, the author, long before the experiments under discussion were published, mentioned that practically all erythrocytes in the pigeon contained reticulum.[8] The same was true, and more exclusively so, of the cells of other animals, where the nucleated erythrocyte is the mature form. Naturally he assumed that all nucleated cells should show a reticulum, and he expressed surprise when in the pigeon, occasionally, he met with an erythrocyte containing no trace of reticulum. A similar statement had been made previously by Key.[9]

When a drop of pigeon's blood is taken on a slide and immediately mixed with a drop of saturated brilliant cresyl blue solution of approximately equal size, and then covered gently with a cover slip, it will be observed that all the erythrocytes show a reticulum. However, the reticulum does not appear immediately as in the case with human cells, but considerable time is required before all the erythrocytes will appear reticulated. The best suitable solution of stain is a saturated solution of brilliant cresyl blue, which is less than 0.5 per cent, in a 0.75 per cent solution of sodium chloride (0.75 per cent solution of sodium chloride gave better results in these studies than the standard 0.85 per cent solution). On these preparations only an occasional erythrocyte will be found void of any trace of reticulum, and this can be demonstrated only after a painstaking search.

If pigeon cells are stained with brilliant cresyl blue in the same manner as human blood, either by the cover slip or slide method, about 20 to 70 per cent of the cells show a reticulum when the films are examined immediately. After counterstaining with the Wright's stain, however, almost all the cells lose their reticulum. The results are approximately the same, whether the blood is taken on a slide and then stirred with the blunt end of a tooth pick, previously dipped into the brilliant cresyl blue solution, and then quickly filmed and dried, or when the blood is taken on a pre-prepared cover slip that has been filmed with

an alcoholic solution of brilliant cresyl blue, dried and polished, this inverted and placed on another cover slip and then pulled apart. The frequency of the reticulated cells on these preparations depends upon the time one takes to spread the blood on the slide. If the preprepared cresyl blue cover slip is fairly well coated with the dye, and considerable time is allowed to lapse before the cover slips are pulled apart, or when the blood-dye mixture is stirred for quite a while before it is filmed, more cells will show the reticulum, especially before counterstaining. After such films are counterstained, another factor has to be considered; namely, the complete removal of the brilliant cresyl blue and its replacement by the methylene blue of the counterstaining material. In a previous paper we[10] have pointed out that even the reticulum of human erythrocytes can be properly and permanently stained only if the vital stain is removed from the specimen and completely replaced by the methylene blue of the counterstaining material. The difficulties encountered in staining human erythrocytes are multiplied during staining of erythrocytes from species of animals where the nucleated red blood cell is the mature form, as is the case in the pigeon. Two factors may be responsible for this difficulty. The covering of the erythrocyte, the "membrane," might be more firm and less permeable, or the reticulum-forming material is less easily precipitated by the vital dyes, such as the brilliant cresyl blue. That this substance is very different from that found in human erythrocytes, it is less likely, since it is precipitated in the same manner by the same substances, forming flakes similar in shape and size with the same stain. But the physical structure of the avian erythrocyte differs from that of the human, or mammalian in general. Whereas the former is elliptic and convex, the latter is round and concave; and whereas the former's "membrane" is pliable and elastic, assuming under certain conditions various shapes and returning to its original shape after such conditions are removed (elongated forms in pernicious and other anemias, sickle-shaped forms in sickle-cell anemia, etc.), the pigeon's erythrocyte breaks under stress, and the two parts do not assume always entirely smooth forms, but continue to exhibit the irregular edges at the site of the breaking. The instant penetration of the brilliant cresyl blue into the human erythrocyte and the delayed staining in the avian cell may point to such a difference in permeability of their respective coverings. If these assumptions are true, then the proper way to stain the pigeon's blood vitally is to expose the cells to a proper solution of the stain, and to allow sufficient time to lapse so that the reticulum-forming substance is completely precipitated. During the counterstaining process it is very important that the slide or cover slip is covered with liberal amounts of the Wright's stain, and after the water is added, sufficient time is allowed to lapse, in order that the methylene blue completely replaces the brilliant cresyl blue. In the experience of this laboratory, three minutes' fixation and five more minutes after the water is added, give the best results.

However, aside from the technical, there is another factor that should be considered in work of the type under discussion. The hemopoietic activity of liver and stomach preparations is limited to pathologic conditions in which, obviously, some factor or factors are missing or deficient. It is not known and

it is not expected that the continuous introduction into the body of a healthy person of such preparations, even in massive doses, will produce an increase in the number of circulating blood cells beyond the natural limits. They are inert even in types of anemias where the hypothetical factor is not deficient or absent. It seems, therefore, unreasonable to expect such substances to stimulate hemopoietic activity in animals, birds, or mammals, when their normal hemopoiesis is not impaired, and bring about just a "reticulocyte response," with or without any expected increase in the number of circulating erythrocytes.

REFERENCES

1. Minot, G. R., and Murphy, W. P.: Treatment of Pernicious Anemia by a Special Diet, J. A. M. A. **87**: 470, 1926.
2. (a) Vaughan, J. M., Muller, G. L., and Minot, G. R.: Response Obtained in Healthy Pigeons by Administration of Substances Effective in Pernicious Anemia, Lancet **1**: 1062, 1930.
   (b) Vaughan, J. M., Muller, G. L., and Zetzel, L.: Response of Grain-Fed Pigeons to Substances Effective in Pernicious Anemia, Brit. J. Exper. Path. **11**: 456, 1930.
3. (a) Edmunds, C. W., Brueckner, H. H., and Fritzell, A. L.: On a Laboratory Test for Liver Extract, J. Am. Pharm. A. **22**: 91, 1933.
   (b) Peabody, W. A., and Neale, R. C.: The Pigeon as a Hematopoietic Test Animal, J. Am. Pharm. A. **22**: 1231, 1933.
   (c) Muller, G. L.: Influence of Liver Extract and Acute Infection on the Reticulocytes and Bone Marrow of Pigeons, Arch. Path. **14**: 774, 1932.
4. Wills, L.: Spontaneous Fluctuations in the Reticulocyte Count in Pigeon's Blood, Brit. J. Exper. Path. **13**: 172, 1932.
5. Heimann, H., Connery, J. F., and Goldwater, L. J.: Lack of Effect of Liver Treatment on the Circulating Reticulocytes in the Pigeon, Am. J. M. Sc. **188**: 343, 1934.
6. Gurd, M. R.: The Use of Grain-Fed Pigeons in the Biological Assay of Liver Preparations, Quart. J. Pharm. & Pharmacol. **8**: 39, 1935.
7. (a) Muller, G. L.: Reticulocyte Responses in the Pigeon Produced by Material Effective and Non-effective in Pernicious Anemia With Description of Histologically Different Reactions of Bone Marrow, New England J. Med. **213**: 1221, 1935.
   (b) (1) Wakerlin, G. E., and Bruner, H. D.: Further Evidence for the Presence of a Toxic Factor in Pernicious Anemia, Science **82**: 494, 1935.
   (2) Modified Pigeon Method for Bioassay of Antipernicious Anemia Liver Extracts, J. Pharmacol. & Exper. Therap. **58**: 1, 1936.
   (3) Presence in Normal Human Urine of Reticulocyte-Stimulating Principle for Pigeon, Arch. Int. Med. **57**: 1032, 1936.
8. Nittis, S.: "Supra-vital" Staining Phenomena of Reticulated Erythrocytes, Folia haemat. **41**: 385, 1930.
9. Key, J. A.: Studies on Erythrocytes, With Special Reference to Reticulation, Polychromatophilia and Mitochondria, Arch. Int. Med. **28**: 511, 1921.
10. Nittis, S.: The Nature and the Mechanism of Staining of the Erythrocytic Reticulum. Am. J. M. Sc. (In press.)

SERUM POTASSIUM IN HYPERTHYROIDISM*

SVEND PEDERSEN, PH.D., WALTER G. MADDOCK, M.D., AND
SHERWOOD WINSLOW, M.D., ANN ARBOR, MICH.

THE condition of thyroid crisis is of considerable concern because it accounts for at least 50 per cent of the deaths from goiter.[1, 2] Generally more than half of this mortality occurs on the medical service before the patient can be improved to the point of even considering an operation. The remainder die from severe postoperative reactions. As indicated by these figures, the treatment is most unsatisfactory, the usual measures being merely supportive in nature. In the hope of learning more about this distressing complication a number of studies on patients with hyperthyroidism have been carried on at the University Hospital.

Among the ions investigated potassium was included for several reasons. Blake[3] first showed potassium to be a toxic substance in 1839. Potassium is reported to be increased in the conditions shown in Table I,[4-17] among them being epinephrine injections. Camp and Higgins[18] have shown that potassium can produce effects in dogs similar to those caused by epinephrine. Overactivity of the adrenal glands has long been thought to be a cause of severe hyperthyroid reactions, and epinephrine has been reported to be increased in hyperthyroidism.[19-23] In preoperative studies on patients with hyperthyroidism, serum potassium has not been found to deviate materially from the normal,[16, 24, 25] and in two patients dying from postoperative hyperthyroid reactions serum potassium was unaltered.[2] Considering the relationship between epinephrine and potassium, it was thought worth while to investigate a more extensive series of patients with hyperthyroidism, particular attention being paid to a possible correlation between the serum potassium findings and the mildness or severity of the postoperative reaction.

PROCEDURE

Serum potassium was determined on the patient soon after admission to the hospital, then a few hours before the operation, and on the first, second, and third postoperative days. The method of Shohl and Bennett[26] was used with minor modifications. Filtrate equivalent to 2 c.c. of serum was taken to dryness in silica beakers and ashed overnight. The ash was dissolved in hot water and transferred to a small pyrex beaker, evaporated again to dryness, and precipitated, filtered, and the color developed in the usual manner. It was felt that the

*From the Department of Surgery, University of Michigan.
Received for publication, January 4, 1938.
This investigation was aided by a grant from the Horace H. Rackham Fund for Graduate Studies.

TABLE I

CONDITIONS IN WHICH SERUM POTASSIUM IS INCREASED

CONDITION	SUBJECT	AVERAGE VALUES MG. PER CENT		REFERENCE
		Before	*After*	
Injection of epinephrine	3 cats	19.8	25.1	D'Silva[4a]
Injection of epinephrine	dogs		+3.0-8.0	Marenzi and Gerschman[5]
Injection of epinephrine	8 rabbits	14.6	21.1	Schwarz[6]
Injection of epinephrine with resulting pulmonary edema	3 rabbits	21.6	39.9	Caccuri[7]
Adrenalectomy	11 cats	19.0	28.0	Baumann and Kurland[8]
Hemolytic shock	8 dogs	20.8	98.3	Ilin and Vavzykovskaja[9]
		Before	*During*	
Histamine shock	5 dogs	18.6	26.7	Kuschinsky[10]
Addison's disease	1 human being	----	34.2	Loeb[11]
Intestinal obstruction	8 cats	24.5	41.5	Scudder, et al.[12]
Artificial fever	2 rabbits	15.9	32.4	Kallós-Deffner[13]
Anaphylactic shock	rabbits			Schittenhelm, et al.[14]
acute shock	carotid artery	25.0	54.6	
	portal vein	29.0	62.7	
	hepatic vein	29.8	60.0	
prolonged shock	carotid artery		38.0	
	portal vein		68.9	
	hepatic vein		57.1	
Experimental shock	cats		+50-100 per cent	Zwemer and Scudder[15]
		During	*After*	
Intestinal obstruction	1 human being	29.8	17.2	Scudder, et al.[12]
Fever	7 human beings	22.2	17.9	Kallós-Deffner[13]
Bright's disease	15 human beings	25.5		Hoffman and Jacobs[16]
Cardiac failure	15 human beings	17.0-36.8		Schnitker, et al.[17]

transfer of the potassium chloroplatinate to the asbestos filter was facilitated in this manner. Considerable care was exercised in taking the blood samples and only nonhemolyzed serum was used in the determinations. These were always run in duplicate, and twice distilled water was used in all manipulations.

TABLE II

SERUM POTASSIUM DATA ON PATIENTS WITH HYPERTHYROIDISM

PATIENT NO.	SEX	B.M.R.	AGE	PREOPERATIVELY			DAY OF OPERATION	POSTOPERATIVELY				POST-OPERATIVE REACTION
								1ST DAY	2ND DAY	3RD DAY	4TH DAY	
				MG. %	MG. %	MG. %	MG. %	MG. %	MG. %	MG. %	MG. %	
1	F	+42	20	21.7	20.3		19.8	18.0	22.0	20.9	22.4	Mild
2	M	+ 4	50	21.1			17.9	19.1	19.4	18.5		Mild
3	F	+36	45	23.8	23.8	21.1	20.6	19.1	21.9	18.0	21.5	Severe thyroid crisis
4	F	+37	39	18.4	19.8		22.2	18.0	17.4	20.1		Mild
5	F	+23	29	22.5			21.1	20.3	17.2	18.4		Mild
6	F	+38	50	19.8	20.5		22.9	21.4	19.1	18 2		Mild
7	F	+ 3	26	18.9	18.6		20.9	18.1	19.7	20.2		Mild
8	F	+26	56	19.4			22.5	19.4	24.1	17.9	23.8	Mild
9	F	+16	50	19.3			20.8	18.6	18.0	16.6		Mild
10	F	+19	35	21.9	22.4		18.5	17.0				Died in thyroid crisis
11	F	+14	37	21.2			20.2	19.2	19.6	21.3	18.1	Mild
12	F	- 6	53	21.3			22.2	21.6	23.5	22.6		Mild
13	M	+ 4	38	24.2			26.1	20.6	21.7	18.4	20.0	Mild
14	M	+28	26	20.9	20.7	23.5	23.9	24.7	24.0	20.2	22.0	Mild
15	F	+36	49	22.2				19.5	20.6			
Average				21.1			21.4	19.6	20.6	19.3		

Average of 10 other patients with hyperthyroidism 21.3 Mg. per cent (Range 17.8-24.7)
Average of 7 control patients (with other diseases) 21.4 Mg. per cent (Range 20.3-22.2)

RESULTS

The data obtained in this study are shown in Table II. Besides the results for the 15 patients followed both before and after the operation, the limits and the average for 10 additional patients with hyperthyroidism studied preoperatively are given for reference, and the limits and average of 7 patients in a control group are given for comparison.

COMMENT

It can be readily seen that the preoperative serum potassium values of the patients with hyperthyroidism were normal (18 to 22 mg. per cent) and that only minor variations occurred in the serum potassium during the postoperative period. The two patients who developed thyroid crises did not vary in this regard from the others.

Unless in hyperthyroidism there should be an increased sensitivity toward potassium similar to that believed to exist in adrenal insufficiency, the concentrations of serum potassium found in this investigation could not be responsible for any toxemia.

SUMMARY

In a study of 15 patients with hyperthyroidism, serum potassium was found to be essentially normal before and after thyroidectomy and during thyroid crises. Ten additional hyperthyroid patients studied preoperatively had normal values.

Apparently serum potassium is not affected by hyperthyroidism and is not concerned with the toxic state of thyroid crisis.

REFERENCES

1. Goetsch, E.: Criteria of Operability for Goiter, Minnesota Med. **18**: 631, 1935.
2. Pemberton, J. de J.: Postoperative Hyperthyroid Reactions, West. J. Surg. **44**: 521, 1936.
3. Blake, J.: Observations on the Physiological Effects of Various Agents Introduced Into the Circulation, as Indicated by the Haemadynamometer, Edinburgh M. J. **51**: 330, 1839.
4. (a) D'Silva, J. L.: The Action of Adrenaline on Serum Potassium, J. Physiol. **86**: 219, 1936.
   (b) D'Silva, J. L.: The Action of Adrenaline on Serum Potassium, J. Physiol. **82**: 393, 1934.
   (c) D'Silva, J. L.: Action of Adrenaline on the Perfused Liver, J. Physiol. **87**: 181, 1936.
5. Marenzi, A. D., and Gerschman, R.: Adrenaline et potassium sanguin, Compt. rend. Soc. de biol. **124**: 382, 1937.
6. Schwarz, H.: Einwirkung des Adrenalins auf den Kaliumgehalt des Blutserums, Arch. f. exper. Path. u. Pharmakol. **177**: 628, 1934.
7. Caccuri, S.: Turbe dell' equilibrio elettrolitico nell edema polmonare acuto da adrenalina, Riforma med. **46**: 1545, 1930.
8. Baumann, E. J., and Kurland, S.: Changes in the Inorganic Constituents of Blood in Suprarenalectomized Cats and Rabbits, J. Biol. Chem. **71**: 281, 1926-27.
9. Ilin, V. S., and Vavzykovskaja, E.: Experimentelle Untersuchungen über das Wesen des hämolytischen Shocks bei der Bluttransfusion. VIII Mitteilung. Über die Ursachen der Steigerung des Kaligehalts im Serum im Anschluss an den hämolytischen Shock nach Bluttransfusion, Arch. f. klin. Chir. **185**: 608, 1936.
10. Kuschinsky, G.: Über das Verhalten von Kalium und Calcium im Blut des Hundes beim Histaminshock, Ztschr. f. d. ges. exper. Med. **64**: 563, 1929.
11. Loeb, R. F.: Chemical Changes in the Blood in Addison's Disease, Science **76**: 420, 1932.
12. Scudder, J., Zwemer, R. L., and Truszkowski, R.: Potassium in Acute Intestinal Obstruction, Surgery **1**: 74, 1937.
13. Kallós-Deffner, L.: Über Kaliumgehalt des Blutserums im Fieber, Acta. med. Scandinav. **85**: 221, 1935.
14. Schittenhelm, A., Erhardt, W., and Warnat, K.: Über den Kalium- und Calciumgehalt von Blut und Organen des Kaninchens und des Hundes und seine Veränderungen beim sensibilisierten und anaphylaktischen Tier, VII. Mittheillung, Ztschr. f. d. ges. exper. Med. **58**: 662, 1928.
15. Zwemer, R. L., and Scudder, J.: Potassium Changes in Experimental Shock, Am. J. Physiol. **119**: 427, 1937. Am. Physiol. Society Proceedings.
16. Hoffman, W. S., and Jacobs, H. R. D.: The Partition of Potassium Between the Serum and Corpuscles in Health and Disease, J. Lab. & Clin. Med. **19**: 633, 1934.
17. Schnitker, M. T., Van Raalte, L. H., and Cutler, E. C.: Effects of Total Thyroidectomy in Man, Arch. Int. Med. **57**: 857, 1936.
18. Camp, W. J. R., and Higgins, J. A.: The Role of Potassium in Epinephrine Action, J. Pharmacol. & Exper. Therap. **57**: 376, 1936.
19. Bröking, E., und Trendelenburg, P.: Adrenalinnachweis und Adrenalingehalt des menschlichen Blutes, Deutsches Arch. f. klin. Med. **103**: 168, 1911.
20. Fraenkel, A.: Über den Gehalt des Blutes an Adrenalin bei chronischer Nephritis und Morbus Basedowii, Arch. f. exper. Path. u. Pharmakol. **60**: 395, 1908-09.

21. Adler, L.: Untersuchungen über den Adrenalingehalt des Blutes, Deutsches Arch. f. klin. Med. **114**: 283, 1914.

22. Maddock, W. G., Coller, F. A., and Pedersen, S.: Thyroid Crisis: Its Relation to Liver Function, West. J. Surg. **44**: 513, 1936.

23. Maddock, W. G., Pedersen, S., and Coller, F. A.: Studies of Blood Chemistry in Thyroid Crisis, J. A. M. A. **109**: 2130, 1937.

24. Kylin, E.: Der Gehalt des Blutes an Calcium und Kalium, Acta med. Scandinav. Suppl. **19**: 1, 1927.

25. Fiedler, A.: Recherches sur les cations du sang dans certains états physiologiques et pathologiques, J. de physiol. et de path. gén. **32**: 1121, 1934.

26. Shohl, A. T., and Bennett, H. B.: A Micromethod for the Determination of Potassium as Iodoplatinate, J. Biol. Chem. **78**: 643, 1928.

NOTE: Ancel Keys, Am. J. Physiol. **121**: 325, 1938, found epinephrine to cause an immediate and marked lowering in plasma potassium in normal man. In twenty minutes the level is back to near normal and rises above normal after forty to sixty minutes.

THE REACTION OF UROBILINOGEN WITH P-DIMETHYLAMINOBENZALDEHYDE*

HANS NORBERT NAUMANN, M.D., BROOKLYN, N. Y.

IN PREVIOUS papers (Naumann, 1936) results appeared of a study of Ehrlich's p-dimethylaminobenzaldehyde test for urobilinogen in urine, and a modification of the test was presented. The nonspecificity of the test was indicated, and it was recommended that it should be confirmed by the use of Schlesinger's zinc acetate alcohol test for urobilin. In the present work the problem of specificity and the course of the urobilinogen aldehyde reaction in different acids have been made the subject of a closer study.

The Nonspecificity of the Urobilinogen Aldehyde Test.—In order to give as complete a survey as possible of substances reacting with p-dimethylaminobenzaldehyde the statements in the literature have been reviewed and supplemented by experiments of the author. The results obtained are summarized in Table I. The second column gives the color obtained in HCl solution, either as performed by the authors cited or by the present author. In these experiments 3 drops of 1 per cent p-dimethylaminobenzaldehyde in concentrated HCl were added to 2 ml. of solution. The third column presents additional information regarding the effect of heat, the addition of sodium acetate, and the performance of the test upon ether or light petroleum extracts of the test solution.

As will be seen from the table, indole, indican, protein derivatives, salvarsan, phenazone, acriflavine, and rhubarb extract are extracted with ether and so can mask a urobilinogen aldehyde test. The same substances as well as urea and novocain give a stable or increased color after addition of sodium acetate.

*From the Long Island College of Medicine Division, Kings County Hospital, Brooklyn. Received for publication, January 6, 1938.

TABLE I

SURVEY OF SUBSTANCES TESTED WITH P-DIMETHYLAMINOBENZALDEHYDE*

SUBSTANCES	COLOR OF REACTION IN HCl	POSITIVE REACTION SPECIAL CONDITIONS OF TEST	NEGATIVE REACTION SUBSTANCES
	A *Substances Excreted Under Normal Conditions*		
Urobilinogen	Red[6, 15, 19, 20]	Stable in HCl + $C_2H_3O_2Na$, positive in ether and light petroleum extract	Uric acid[1]
Indole	Red[19, 20]	Red or yellow in HCl + $C_2H_3O_2Na$, colorless in ether extract, reddish in light petroleum extract	Creatine[1]
			Creatinine[1]
Skatole	Blue[19, 20]		Cholesterol
Indican	Yellow to brownish	Stable in HCl + $C_2H_3O_2Na$, positive in ether and light petroleum extract	
Phenol	Yellowish to reddish orange[20]	Only after heating	
Urea	Yellow[1]	Stable in HCl + $C_2H_3O_2Na$	
Allantoin	Yellowish[1]	Increase after heating	
	B *Substances Excreted Under Pathological Conditions*		
Mesobilirubinogen	Same as urobilinogen[9, 13]	Same as urobilinogen	Urobilin[20]
			Bilirubin[20]
			Hematin[20]
Tryptophan	Yellowish to reddish[17]	Only after heating	Hematoporphyrin[20]
Protein and protein derivatives	Reddish to orange violet[15, 17, 19]	Stable in HCl + $C_2H_3O_2Na$, positive in ether and light petroleum extract	Tyrosine[20]
			Leucine[1, 15]
			Cystine[1]
Sodium hydroxybutyrate†	Pink	Fading quickly, faint yellow in HCl + $C_2H_3O_2Na$, fading quickly in ether extract	
Acetone	Conc.: green Dil.: pink[2]	Fading if diluted, fading in HCl + $C_2H_3O_2$ Na	
	C *Drugs*		
Morphine	Reddish orange }	Only after heating, decreasing in HCl + $C_2H_3O_2Na$	Codeine
Camphor	Yellowish orange }		Caffeine
			Atropine
		Increase in HCl + $C_2H_3O_2Na$	Suprarenine
Novocain	Yellow[4]	Stable in HCl + $C_2H_3O_2Na$, positive in ether and light petroleum extract	Quinine
Neosalvarsan	Yellow		
Phenazone (Antipyrine)	Salmon colored[5]	Stable in HCl + $C_2H_3O_2Na$, positive in ether extract	Amidopyrine (Pyramidon)
Phenacetin	Yellowish	Only after heating, increase in HCl + $C_2H_3O_2Na$, reddish precipitation after standing	Acetylsalicylic acid

TABLE I—CONT'D

Rhubarb extract	Brownish	Stable in HCl + $C_2H_3O_2Na$, positive in ether extract	Senna extract
Phenylazo-2-6-diaminopyridine monohydrochloride (Pyridium)	Red[7]	In HCl without aldehyde	Senega extract Ipecacuanha extract
Trypaflavine	Light brown to dark red[11]	Red pigment not extracted by chloroform	
Acriflavine (Diaminomethyl-acridinium-chloridehydro-chloride)	Red	Brown to yellow precipitation in HCl + $C_2H_3O_2Na$, faint red in ether extract	

D *Chemical Compounds*

Dimethylpyrrole carbonic acid Phenyl-dimethylpyrrole carbonic acid Dimethylpyrrole	Colorless crystals	Dry heating and addition of $KHSO_4$[8]	Serine[15, 17, 20] Lysine[15, 17, 20] Arginine[15, 17, 20]
Reduced hematin[15] Reduced hematoporphyrin[18] Hemopyrrole[18] Acetylated glycosamine[5*‡] Dimethylpyrrole acetic acid[20]	Red		Aminovaleric acid[15, 17, 20] Proline[5, 15, 17, 20] Phenylalanine[15, 17, 20] Glucosamine[5]
Carboxylated cryptopyrrole carbonic acid and derivatives	Colored crystals	In perchloric acid[10]	Parabanic acid[1] Barbituric acid[1]
Phenylmethyl-pyrazolone	Red substance[18]		Bromdiethyl-urea (Adalin)[1]
Asparagine Glutamine	Orange[1]	Slow reaction in HCl without aldehyde	Bromisovaleryl-urea[1] (Bromural)
Glycocoll Alanine Allantoin Biuret	Faint yellow[1]		Purine Guanidine } Derivatives[1]
Glycocholic acid	Blue, rapidly fading[1]		
Aniline	Yellow	In HCl without aldehyde	Histidine[17] Histamine
Pyridine[20]	Yellow§	Decrease in HCl + $C_2H_3O_2Na$	
Toluene	Reddish	Stable in HCl + $C_2H_3O_2Na$	Hydroquinone
Resorcin	Pink		Vanillin
Benzidine	Red jelly		
Sulfanilic acid	Yellow		
Phenylhydrazine	Yellow	Reddish orange in HCl without aldehyde	

*The superior numbers refer to the references at the end of the paper. Data without reference numbers represent author's experiments.
†A preparation kindly given by Professor A. Magnus-Levy, Berlin.
‡After treatment with alkali.
§Thomas states a blue color which could not be observed.

Among substances interfering with Schlesinger's test, porphyrines, flavine compounds, and eosin are to be mentioned, all of which already exhibit a greenish fluorescence without the addition of zinc acetate alcohol. The faint reddish fluorescence of bilirubin in the presence of zinc acetate alcohol and iodine can be avoided by using potassium permanganate for oxidation instead of iodine.

Urobilinogen Aldehyde Test in Different Acids.—The aldehyde test can be performed also in the presence of weak acids, e.g., formic, acetic, butyric, trichloracetic, lactic, tartaric, and boric acid. A reaction with these weak acids can be obtained only in concentrated solutions dissolving the residue of an urobilinogen extract and the p-dimethylaminobenzaldehyde directly within the acids. The rate of reaction is generally proportional to the acidity, so that the rapidity of the reaction decreases as follows: formic = tartaric > acetic > boric acid. The reaction in boric acid takes several days and appears only as reddish pigment on the surface of the crystals. The reaction with pure indole is similar.

It has been observed, furthermore, that the reaction can be accelerated by the addition of water or iodine. Conditions are similar using concentrated HCl in the residue of the ether or light petroleum extracts or in the extract itself, provided it is thoroughly dry. Indole, indican, and tryptophan, however, react somewhat differently (see Table II), inasmuch as a color is obtained with concentrated HCl alone which changes after the addition of water.

TABLE II

ALDEHYDE REACTION OF INDOLES IN SUBSTANCE WITH CONC. HCl

	CONC. HCl + P-DIMETHYL-AMINOBENZAL-DEHYDE	ADDITION OF H_2O	ADDITION OF IODINE	CONC. HCl ALONE	ADDITION OF H_2O	ADDITION OF IODINE
Indole	Yellow	Red	Red-brownish precipitation	Faint yellow	Colorless	Yellow darkening
Indican	Reddish	Yellow	Dark brown precipitation	Yellow	Yellow	Blue
Tryptophan	Violet blue	Red violet	Yellow brown cloudy	Colorless	Colorless	Yellow (iodine color)
Urobilinogen	Colorless	Red	Reddish	-	-	-

A spectroscopic study of the urobilinogen aldehyde band at 557 $\mu\mu$ and the urobilin band at 490 $\mu\mu$ using different acids revealed the presence of a system of three concurrent reactions:

(1) The actual urobilinogen-aldehyde reaction.

(2) The oxidation of urobilinogen into urobilin before reacting with benzaldehyde.

(3) The decomposition of the urobilinogen-aldehyde compound, partly into urobilin, partly into unknown products.

These conditions can be illustrated by the following scheme:

$$\text{Urobilin} \leftarrow \text{Urobilinogen} \rightarrow \left[\begin{matrix}\text{Urobilinogen} \\ \text{p-dimethylaminobenzaldehyde}\end{matrix}\right] \rightarrow \begin{matrix}\text{Urobilin} \\ \searrow\ \text{Unknown decomposition products}\end{matrix}$$

The decisive factor in the equilibrium of the system is the rapidity of the urobilinogen-aldehyde formation, a shifting to the left side taking place in weak acids and to the right side in strong acids. An accelerating influence by heat is present to a certain extent. An advantageous combination of both tendencies is reached in Terwen's method by application of initial strong and subsequent weak acid. Even then the result is not complete, since a urobilin band can always be observed as noted also by Terwen himself (1925) and confirmed later by Heilmeyer (1931). Recently Watson (1936) determined that the total loss of urobilinogen, including that which occurs during the reduction of urobilin with ferrous sulfate, amounts to 13-30 per cent, which is allowed for in calculation.

SUMMARY

1. A complete survey has been given of physiological substances, drugs, and chemical compounds reacting with p-dimethylaminobenzaldehyde.

2. The role of acidity in the urobilinogen-aldehyde test has been studied, with special reference to weak acids.

I am indebted to Dr. Tasker Howard, Director of the Long Island College Division, Kings County Hospital, Brooklyn, and to Dr. C. H. Greene, Clinical Professor of the Long Island College of Medicine, for their help and interest in my work. I wish to record also my thanks to the Emergency Committee in Aid of Displaced Foreign Physicians for a personal grant.

REFERENCES

1. Barrenscheen, H. K.: Ueber eine Reaktion des Harnstoffs mit p-Dimethylaminobenzaldehyd, Biochem. Ztschr. **140**: 426, 1923.
2. Brocher, J. E. W.: Täuschungsmöglichkeiten bei Anstellung der Ehrlich'schen Dimethylparamidobenzaldehydreaktion im Harn, Schweiz. med. Wchnschr. **62**: 601, 1932.
3. Charnas, D.: Uber die Darstellung, das Verhalten und die quantitative Bestimmung des reinen Urobilins und des Urobilinogens, Biochem. Ztschr. **20**: 401, 1909.
4. Christophe, G.: Le dosage de l'urobiline urinaire, J. Pharm. Chim. **19**: 105, 1934.
5. Clemens: Zur Ehrlich'schen Dimethylaminobenzaldehydreaktion, Deutsches Arch. f. klin. Med. **71**: 168, 1901.
6. Ehrlich, P.: Uber die Dimethylamidobenzaldehydreaktion, Med. Wchnschr. **13**: 151, 1901.
7. Farthing, J. W., and Beck, J. S. P.: False Ehrlich Reaction Caused by Pyridium Medication, J. Lab. & Clin. Med. **20**: 61, 1934.
8. Feist, F.: Uber Kondensation von Pyrrolen und Pyrrolcarbonsäureestern mit aromatischen Aldehyden, Ber. Dtsch. Chem. Ges. **35**: 1647, 1902.
9. Fischer, H., and Meyer-Betz, F.: Zur Kenntnis der Gallenfarbstoffe, Ztschr. f. physiol. Chem. **75**: 232, 1911.
10. Fischer, H., and Nenitzescu, C.: Synthese der carboxylierten Kryptopyrrolcarbonsäure und einiger Methene, Ztschr. physiol. Chem. **145**: 295, 1925.
11. Goldschmidt, R.: Zur Spezifität der Ehrlichschen Aldehydreaktion im Harn, Deutsche med. Wchnschr. **56**: 140, 1930.
12. Heilmeyer, L.: Die spektrometrische Bestimmung des Urobilins und Urobilinogens, Ztschr. f. d. ges. exper. Med. **76**: 220, 1931.

13. Lemberg, R.: Urobilinogen, Nature **134**: 422, 1934.
14. Naumann, H. N.: Studies on Bile Pigments, Biochem. J. **30**: 347 and 1021, 1936.
15. Neubauer, O.: Uber die Bedeutung der neuen Ehrlichschen Farbenreaktion, Sitzungsber. Ges. f. Morph. u. Physiol. 1903, Juliheft.
16. Niemann, C.: Der Absorptionskoeffizient des Mesobilirubinogens und Koproporphyrins, Ztschr. f. physiol. Chem. **146**: 181, 1925.
17. Rhode, E.: Die Farbenreaktionen der Eiweisskorper mit p-Dimethylaminobenzaldehyd und andern aromatischen Aldehyden, Ztschr. f. physiol. Chem. **44**: 161, 1905.
18. Steiner: Uber das p-Dimethylaminobenzaldehyd, Diss. Phil., Berlin, 1904.
19. Terwen, A. J. L.: Uber ein neues Verfahren zur quantitativen Urobilinbestimmung, Deutsches Arch. f. klin. Med. **149**: 72, 1925.
20. Thomas, K.: Urobilinogen, Diss. Freiburg, 1907.
21. Watson, C. J.: Studies of Urobilinogen, Am. J. Clin. Path. **6**: 458, 1936.

DIFFERENTIAL DIAGNOSIS OF MULTIPLE MYELOMA AND HYPERPARATHYROIDISM BY MEANS OF BIOPSY*

W. Warren Sager, M.D., Roger M. Choisser, M.D., and G. Louis Weller, Jr., M.D., Washington, D. C.

THE differential diagnosis of multiple myeloma and hyperparathyroidism is frequently a conjecture until the late stage. Both conditions are insidious in onset, requiring several months and occasionally years before the characteristic symptoms and physical findings are manifested. In typical cases of hyperparathyroidism it is obviously not difficult to make a diagnosis when classic symptoms, such as severe bone pains, high blood calcium associated with pathologic fractures, and x-ray evidence of multiple bone cysts are present. This is likewise true in multiple myeloma where the lesions shown in the roentgenogram have the characteristic distribution and appearance. However, in some instances, the symptoms, physical findings, and x-ray pictures merge so closely that a differentiation is impossible, except by means of direct biopsy, either of the pathologic gland itself or of one of the bone lesions. The case in question was of this indeterminate type and accordingly is reported as one in which the correct diagnosis was made by biopsy of the bone lesion.

REPORT OF CASE

B. E. H., a 50-year-old white male, felt a "little under par" in July, 1936. Upon consulting a physician at that time, a trace of albumin was found in the urine. X-ray of the teeth just prior to this showed two to be infected; these were removed. Still feeling unimproved the patient returned to his physician four months later, only to note that upon repetition of the urinalysis, a moderate amount of albumin was still present. Because of this albuminuria, several more teeth were extracted. The last extraction occurred on July 5. By August 1, the gum not having healed and there being an odor from that area, a return visit

*From the Departments of Surgery, Pathology, and Medicine, School of Medicine, the George Washington University, Washington, D. C.
Received for publication, January 6, 1938.

to the dentist resulted in removal of granulation tissue and packing. Meanwhile the patient's kidneys were assumed to be infected, and subsequently the diagnoses of rheumatic fever and bacterial endocarditis were entertained, but unsuccessfully.

Meanwhile the patient continued to become worse. Weight loss by this time was 35 pounds, and twitchings in the leg muscles began. With sedatives, including opium, and a high caloric diet, the patient's twitchings were relieved. He began to gain weight and walked from one to two miles daily. This improvement stopped, however, after three to four weeks. Weakness, pains in the chest and back then began. The patient emphasizes particularly that he could pinch the skin over the chest without pain, but that pressure on the ribs caused pain. X-rays of the chest taken at this time showed a generalized decalcification of the bones, together with cystlike areas in the ribs. Additional x-rays of skull showed a moderate amount of mottling with several clear-cut, punched out areas. The spine x-rays revealed gross deformity due to compression of the lumbar vertebra, while throughout the pelvis were many cystlike areas. Gastrointestinal studies revealed a deformity of the duodenum, but no ulcer crater was visible. Anemia was marked. A diagnosis of hyperparathyroidism was made.

Fig. 1.—Lesion in rib utilized for biopsy.

Treatment was carried along two lines. He was given x-ray over the parathyroid region as well as three treatments over the trunk for generalized pruritus. Response was good in that following the second treatment a considerable portion of the bone tenderness disappeared. On the other hand, the continued high caloric, high protein diet soon became associated with high nonprotein nitrogen values. These were accompanied by considerable nausea and an increase in muscular twitchings. Following hospitalization and a low protein diet, with considerable fluids, the patient soon improved. Additional therapy included calcium, iron, and liver. Erythrocytes on February 15 were 1.6 millions, hemoglobin 31 per cent, and leucocytes 4.8 thousands. Following a transfusion on February 27, erythrocytes rose to 2.2 millions, hemoglobin to 44 per cent, and leucocytes to 4.8 thousands. While the patient was under the care of a private physician at home, the protein in the urine was found to be of the Bence-Jones type.

The above history was obtained when the patient was seen by one of us for the first time on March 20, 1937. A review of the systems revealed the following symptoms at that time: The patient feels well in the morning upon awakening but tires considerably from midafternoon onward. There have been slight epistaxes at intervals. He had a moderate amount of dyspnea but no orthopnea. Canker sores occur at irregular intervals. There is pain in the right lower costal margin. Epigastric distress occurs at intervals.

Physical examination revealed a poorly nourished, somewhat ill appearing white male. Skin was very pale, as were the mucous membranes, and a slightly icteric tinge was noted. Tongue was normal. Respirations were slightly labored. Heart was 11 cm. to the left in the fifth interspace, rate 100 to 110. No shocks, thrills, or murmurs were present. Blood pressure was 120 mm. of mercury systolic, 95 diastolic. Veins appeared full. The right kidney was palpable, but of normal size, while no other organs nor masses were palpable in the abdomen. Cervical, supraclavicular and inguinal lymph nodes were normal, while the epitrochlear lymph nodes were palpable.

Fluoroscopic examination of the chest revealed cardiac enlargement, slight bronchiectasis at the right lung base, aorta normal, and no evidences of fluid at the costophrenic angles. Erythrocytes numbered 1.69 millions of cells, leucocytes 4.7 thousands, and 6.5 grams of hemoglobin (Haden-Hausser method). Urine was of a clear yellow color, specific gravity 1.005, acid reaction, no sugar, large amount of albumin (7 grams per liter, Esbach method). Microscopically the urine sediment showed 2 to 3 granular casts and 2 to 3 erythrocytes per high power field, together with an occasional leucocyte. Basal metabolic rate determination

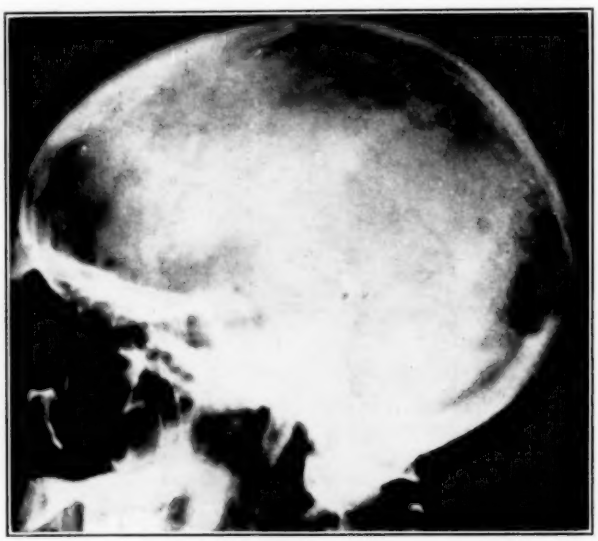


Fig. 2.—Punched out lesions in skull.

on March 21 was plus eighteen. Phenolsulphonephthalein test on March 24, dye injected intravenously, showed less than 5 per cent excretion (estimated) in 60 c.c. of urine the first hour, and even lesser amount in 100 c.c. of urine the second hour.

Continuation of the low protein diet, together with calcium and iron medication, produced little change in the patient. Nonprotein nitrogen, 108 mg. per cent on March 22 dropped to 83 mg. per cent two days after a transfusion on April 1, but by April 6 had returned to 108 mg. per cent. A second transfusion of 500 c.c. on April 15 made little additional change in the patient. On April 14 blood chemistry values were as follows: serum calcium 10.4 mg. per cent, serum albumin 4 gm. per cent, and serum globulin 3.7 gm. per cent.

Because of apparent improvement at intervals with loss of ground at other times, associated with marked discomfort from bone pains and severe cramps in the lumbar region, the possibility of an exploratory operation for a possible parathyroid tumor was considered. Upon reviewing the data in favor of myeloma and of hyperparathyroidism, opinions among roentgenologists were found to be divided about equally in favor of each. It was suggested by one of us (W. W. S.) that biopsy of a bone lesion be done, as a measure which is easier surgically and perhaps much more accurate than search for abnormal parathyroid glands.

With this objective in mind, the tenth rib posteriorly, at the site of a lesion shown in the x-ray, was exposed under local anesthesia April 26, 1937. The rib in this area was enlarged while the surface of the bone was irregular. Removal of the cortex showed a grayish red tissue, which in films stained by Wright's method showed the lesion to consist entirely of plasma cells. The pathologic report of the tissue before fixation and after staining with H. and E. was as follows:

Gross Description.—The specimen consists of a portion of the cortex and bone marrow from the tenth rib. The cortex measures 2 cm. long and 2 mm. in thickness. Attached to the under surface is a small hemorrhagic nodule, which is soft in consistency and measures 5 mm. in diameter.

Microscopic examination of sections made after decalcification shows the cortex thin and eroded, with no evidence of osteogenesis in any area. Osteolysis, however, is noted in several portions. The underlying marrow consists of a diffuse mass of large oval cells loosely held together with delicate fibrillae. The cytoplasm is generous in amount and acidophilic in reaction. The nuclei are large, hyperchromatic, and show radial striations. Mitoses in various phases of division are present throughout. The specimen is typical of a primary tumor of the bone marrow, plasma cell multiple myeloma type.

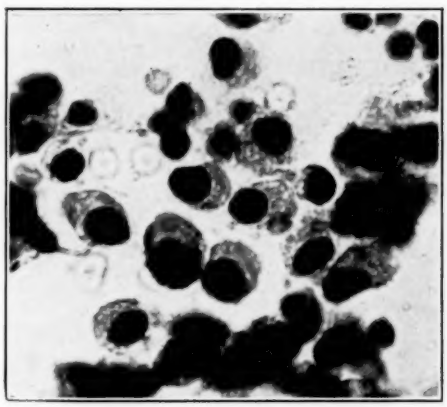


Fig. 3.—Plasma cells in biopsy specimen.

DISCUSSION

The lesions in the bones as shown by x-ray were varied, but for the most part the pelvis, ribs, and skull were involved. In some locations the lesions were believed by several consulting roentgenologists to be typical of hyperparathyroidism. Others interpreted them as being more of the nature of multiple myeloma. One or two areas in the skull were punched out in appearance, but for the most part even in the skull the areas were mottled showing an occasional bone cyst. In the ribs particularly, the process was exceedingly suggestive of the cystic type of lesion which one finds in hyperparathyroidism.

As regards the chemical examination of the blood an initial blood calcium determination is said to have revealed a value of 14 mg. per cent, but subsequent determinations remained around 10 mg. per cent. Serum albumin initially was high, but this subsequently returned to a normal level. Bence-Jones protein constituted practically all of the albumin in the urine, and the amount excreted has remained practically constant. Demonstration of Bence-Jones protein has been successful at intervals. With a fresh specimen of urine it was difficult to demonstrate re-solution of the precipitate as heating

continued unless the urine was strongly acid or alkaline. If nitric acid was used to acidify the urine, the solution took on a reddish purple hue, which was believed to indicate decomposition of the Bence-Jones protein.

When the biopsy of the rib bone marrow was done, the site chosen was a lesion in the tenth rib posteriorly. Prior to operation the possibilities of predisposition to postoperative fracture and nonunion were considered. However, we felt the information to be gained far outweighed any damage which might result. Our fears in this respect were unfounded as the wound healed perfectly after three weeks, with no tendency toward pathologic fracture in this area.

At the time of operation films of bone marrow made from the area near the lesion showed only an immature, cellular marrow, which was normal. Sections taken from the site of the lesion showed the neoplastic process, which consisted of dense masses of plasma-like cells, to be confined to the areas indicated on the x-ray film. The surrounding bone marrow was uninvolved. In view of the above pathologic findings the site of a biopsy must be chosen with this point in mind. The correct diagnosis in this case was made rapidly, without difficulty and with practically no inconvenience to the patient.

Since discharge from the hospital on April 13, 1937, the biopsy site healed by second intention.

The patient continued on his downhill course and was last seen on September 30, 1937, at which time roentgen rays showed progression of the original lesions of the bone marrow. He has moved since to another city and communication with his family on January 3, 1938, revealed that at times he still is able to be semiambulatory, but objectively failing.

SUMMARY

The results of biopsy, in the differentiation of multiple myeloma from hyperparathyroidism in a case presenting features of both diseases, are reported and discussed. Clinical and roentgenologic similarities of these two diseases are easily differentiated by examination of the bone marrow *at the site of one of the lesions.* Biopsy in cases of this type saves much speculation and directs early therapeutic management into proper channels.

The roentgenologists who saw these films and to whom thanks are due for opinions are as follows: Dr. Merrill C. Sosman, of Boston; Doctors F. O. Coe, T. A. Groover, and Claude Moore, of Washington, D. C.

THE CLINICAL SIGNIFICANCE OF EOSINOPHILIA*

ROBERT C. KIRK, M.D., COLUMBUS, OHIO

THE eosinophile is a motile bilobed, rarely trilobed, cell packed with spherical acidophile granules which give the oxidase, peroxidase, and phenophilic reactions and contain ionizable iron. Since the first clear descriptions of the white cell types were made by Ehrlich in 1880,[1] the study of the blood and its cellular elements has become a field of its own in which some of the most progressive research has taken place. Nevertheless, the function of the eosinophile cell and the mechanism of its production remain among the most obscure questions in hematology. Even its origin has been subject to much controversy. An attempt has been made in this paper to review the pertinent literature on these questions and, with the addition of some further clinical material, to arrive at some conclusions regarding them.

Ehrlich[1] believed that all the eosinophiles were formed and multiplied in the bone marrow and were released from there into the circulating blood as a distinct type of cell. Brown[2] and his colleague, Thayer,[3] believed that the characteristic granules were toxic substances absorbed by the neutrophiles. Weidenrich[4] believed they were phagocytosed red blood cell granules. Indeed, Mayr and Moncorps[5] show two photomicrographs of this action taking place in the blood of splenectomized guinea pigs but were unable to demonstrate similar action in human blood. Browning[6] found eosinophiles in the connective tissue of the liver and thymus of ten weeks' old human foetuses, which he believed to occur before the formation of bone marrow, thus disagreeing with Ehrlich and giving rise to a third school, namely, that of Downey,[7] who taught that blood eosinophiles and tissue eosinophiles came from two different sources, a cytometaplasia in situ. Biggart[8] has recently summarized these views and comes to the same conclusion as Bunting[9] that the weight of modern experimental work supports the belief of Ehrlich.

It was thought that some light might be thrown on the function and mechanism of the production of the eosinophile by reviewing the causes of eosinophilia. A heterogeneous series of pathologic substances is known to be associated with a local eosinophilia; e.g., asthmatic sputum (Von Noorden, 1892[10]), nasal secretions in allergic rhinitis,[11] pleural effusions,[12] stools from cases of trichinosis,[13] conjunctival secretions in vernal conjunctivitis,[14] Hodgkin's disease,[15] and the various dermatoses in which sections of the affected tissues show local collections of eosinophiles.

*From the New York Hospital and Department of Medicine, Cornell University Medical College, New York.

Received for publication, January 6, 1938.

Pathologic conditions associated with blood eosinophilia greater than the estimated normal daily fluctuations of from 0 to 4 per cent[16] are leucemia (Ehrlich, 1880[1]);* asthma (Von Noorden, 1892[10, 17-19]); postfebrile states (Zappert, 1892[20-22]); intestinal parasites such as Ankylostoma duodenalis (Müller, 1891[24]); ascaris, taenia, oxyuris (Bücklers, 1894[23]); trichina (Brown, 1897[2]); Filaria bancrofti (Calvert, 1902[25, 26]); Bilharzia haematoba (Coles, 1902[27]); Entamoeba histolytica;[26] echinococcus infection;[29] and Strongyloides stercoralis (Hinman, 1937[28]); periarteritis nodosa (Datnowski, 1909[30, 31]); scabies (Schamberg, 1912[32]); dermatitis herpetiformis;[32] mycosis fungoides;[32] pemphigus;[32] Duhring's bullous disease;[33] eczema;[5, 32] eosinophilic leucemia (Stillman, 1912[35-41]); Simmond's disease (Reiche, 1918[71]); polymyositis (Bittorf, 1919[34, 42]); hay fever (Sternberg, 1928[47]); syphilis;[44] transitory lung infiltration (Löffler, 1932[43]). Several reports[46, 48] of very high blood eosinophile percentages (50 per cent and 90 per cent) in Hodgkin's disease have been found. As early as 1892 Kanthack[45] reported his failure to find them in his own cases. For many years it was believed that the green color of chloromas was due to eosinophilic infiltration; however, Brannan,[51] in a complete review of all cases reported at the time of his paper, was able to find no evidence for this and indeed in the most recent 46 cases of this tumor reported, only one case[74] was found to have an elevated blood eosinophilia. A rare condition of eosinophilia not apparently associated with any known pathologic condition is that of familial eosinophilia (Klinkert, 1911[49, 50]).

In the experimental field a similarly heterogeneous list of procedures has been reported as causing blood eosinophilia in either experimental animals or man such as: oral administration of foreign proteins;[52, 53] parenteral administration of foreign proteins;[8, 54, 55, 58] parasitic infestation;[59] parenteral worm extracts;[5, 58, 60] raw liver diet;[61] insulin;[62] digitalis;[63, 64] bichloride of mercury;[65] camphor and nirvanol intoxications;[54, 56, 57] partial asphyxia;[66, 67] parasympathetic stimulation with drugs;[68] and splenectomy.[19, 72, 73]

OBSERVATIONS

In an attempt to confirm some of the statements made about the various pathologic conditions causing eosinophilia several cases were studied from the records of the New York Hospital since September, 1932.

The literature on the eosinophilia of parasitic infestations is enormous, and its presence is unquestioned. There were 29 cases of trichinosis in this hospital whose blood eosinophiles averaged 35 per cent in the total series, ranging from 4 per cent to 88 per cent, with no patient having less than 16 per cent at some time during hospitalization. In two patients who died, with autopsy confirmation, the blood eosinophilia in one case examined only once showed 20 per cent eosinophiles, and in the second case it was 41 per cent on the day of admission and, at death six days later, had risen steadily to 52 per cent. A third case dying from acute trichinosis, which I have been permitted to report through the kindness of Dr. C. Joseph DeLor from the Medical Service of the University

*Names and dates in parentheses refer to original contributions made to the literature.

Hospital in Columbus, Ohio, had a blood eosinophilia of 64 per cent on the day before death. Opie[59] pointed out in 1904 that there was a definite falling off of the blood eosinophiles in his guinea pigs which were the most heavily infested with trichina spiralis. Since then, many clinicians[70] have felt that a decrease in eosinophiles during the acute stage of the disease was a sign of poor prognosis. This may be true, but as Grace[75] points out in *Pemphigus*, it is probably the result of secondary infection, and fatal outcomes occur without an eosinophile drop.

Eosinophilia occurring in dermatoses of various kinds is second to parasitic diseases in frequency and amount. In 15 cases of various types of pemphigus there was an average blood eosinophilia of 19 per cent. However, the range was from 0 to 22 per cent. Five of these cases died with an average eosinophile count preceding death of 9.8 per cent and a range of 2 per cent to 18 per cent. There is apparently no diagnostic or prognostic significance to these figures. Grace[75] has reported two of these cases and shows that when the eosinophiles fall it is usually coincident with a beginning blood stream infection and is thus a sign of poor prognosis. A similar number of 15 cases, of secondary syphilides, were investigated and found to have an average eosinophilia of 3 per cent, with a range of 0 to 12 per cent. There was no correlation possible between the amount of rash and the eosinophilia.

Typhoid and scarlet fever[20-22] have for many years been considered the best examples of an eosinophilia occurring in the postfebrile states. The series of scarlet fever cases is admittedly very small (9 cases); however, they were all in individuals over 12 years of age, thus avoiding the more marked lability of the eosinophile cell known to occur in young children. No conclusions may be drawn from them except to state that four of the patients had at no time more than 5 per cent eosinophiles noted in their blood smears, and no rhythm was found such as Friedman[22] found in his cases where the eosinophiles reached a peak on the fourth to the seevnth day after the onset of symptoms, falling to normal and again reaching a peak on the twenty-eighth day.

The findings in the 25 cases of typhoid fever seemed to be of more possible significance. Upon admission to the hospital the average blood eosinophilia was less than 1 per cent (0.4 per cent), 2 per 100 cells maximum. The count made preceding discharge from quarantine showed an average of 0.7 per cent, with again a maximum count of 2 per cent. Nine of the 25 cases showed no eosinophiles during their entire hospitalization. Fifteen of the cases had positive blood cultures, all had positive Widals, and three died. It seems reasonable to conclude that there is no notable postfebrile rise of eosinophiles in typhoid fever. Rather does there appear to be a suppression of eosinophiles which may possibly be of some diagnostic importance, certainly as contrasted with trichinosis with which it is clinically occasionally confused.

The occurrence of hypereosinophilia after splenectomy[72, 73] has been investigated in 30 cases in which this procedure has been carried out. Only 13 cases were followed long enough (one year) with interval blood counts to be of significance. Of these, 8 showed no postoperative blood changes. However, 5

showed definite eosinophile increases. For example, one patient, G. C., with Banti's disease whose preoperative eosinophile level did not exceed 4 per cent, had 11 per cent five months after splenectomy, 14 per cent at fifteen months, and 9 per cent at twenty months. These increases occurred as early as the first six weeks after operation and persisted as long as thirty-eight months.

DISCUSSION

It would appear from a study of these pathologic conditions causing eosinophilia that it occurs as a rule in diseases in which the element of protein sensitization or allergy can be introduced. Experimentally it would appear possible to arrive at some conclusion concerning the mechanism of its production.

Probably the simplest experiments producing eosinophilia were those of Opie[52] and Rous.[53] Opie working with guinea pigs and Rous with dogs showed that following periods of starvation the number of eosinophiles in the blood and the thoracic duct lymph was greatly reduced. After the ingestion of protein foods, the number of eosinophiles was greatly increased. This is of some clinical significance inasmuch as Meulengracht and Holm[61] have demonstrated an eosinophilia of from 20 per cent to 70 per cent in pernicious anemia patients eating raw liver, noted about four weeks after beginning the diet. Opie's experiments were in preparation for his later work on the artificial infestation of guinea pigs with trichinae spiralis, thus reproducing the disease as it is found in man.[59] Following this fundamental work, several investigators[5, 58, 60] injected extracts of various worms into animals. Herrick[60] injected daily saline suspensions of ground-up ascaris intraperitoneally into guinea pigs without any noticeable eosinophile effects. However, when similar injections were made at five- to twenty-five-day intervals, striking eosinophile rises occurred. Protein-free filtrates and amino acids so injected produced no changes, leading to the conclusion that this is a protein sensitization, with its refractory periods corresponding to those of "ordinary anaphylaxis." Weinberg and Séguin[58] and Mayr and Moncorps[5] made similar observations. In addition, the former authors were able to show that eosinophiles possess important phagocytic properties with respect to both bacteria and cells. Using hydatid fluid mixed with a suspension of cells rich in eosinophiles, they demonstrated that those eosinophiles which had absorbed toxic material lost their phagocytic properties while these properties were retained by the neutrophiles. In addition, the hydatid fluid lost its antigenic power to produce a positive complement fixation reaction.

Space does not permit a review of all the work done on the parenteral administration of foreign proteins, but the significant findings of several authors[8, 54, 55] will be briefly outlined. Biggart[8] showed that an intramuscular injection of foreign protein gives a polymorphonuclear leucocytosis about the site within twenty-four hours, but at the end of about eight days this reaction is predominantly eosinophilic. Sterile necrosis from a turpentine injection gives similar findings. The injection of lactic acid fails to bring about these changes. If the protein injections are repeated at three-day intervals, there is a definite increase in blood eosinophilia following the second or third injection,

with progressive rises thereafter. These findings support the suggestion that the aseptic destruction of tissue in the animal body will set free factors, probably protein in nature, chemotactic for eosinophile cells. This would appear to be the explanation for their presence in abundance in so many tumors rather than to the presence of lactic acid, which had been suggested by Battaglio.[84]

Campbell, Drennan, and Rettie[54] were unable to obtain a blood eosinophilia before six days using the interval injection method, but they admit that an allergic response may occur in as little as forty-eight hours, which explains Biggart's findings of eosinophile rises on the day of the second injection. This eosinophilia does not seem to be a protective mechanism against foreign protein per se because the presence of a high blood eosinophilia does not alter the allergic or anaphylactic reaction known to occur in sensitized guinea pigs following subsequent doses. Nor is it likely that it is an anaphylactic reaction because the eosinophile changes took place with no relation to the manifestation or lack of manifestation of general anaphylaxis. Using intradermal foreign proteins similar findings were obtained locally; that is, unless allergic reactions took place there was no local eosinophilia. These findings are in accord with the clinical work of Vaughn,[55] who noted that in 18 girls whose blood eosinophilia averaged 1.5 per cent, a maximum of 3.1 per cent was reached on the fourth day after the third injection of Dick toxin, but there was no relation between the severity of the reaction or of the rash to the eosinophilia.

The clinical occurrence of pleural eosinophilia and its explanation may aid in formulating some idea about the cause of eosinophilia. It is estimated to occur in 1 per cent to 5 per cent of all effusions. In this hospital no single instance has been found in 85 cases which have been reported[76] or in an additional 75 cases subsequently examined. Bayne-Jones[12] believes this eosinophilic exudate is due to the eosinotactic influence of protein-split products in an exudate undergoing absorption. This has also been advanced as the explanation for the frequently observed disappearance of blood eosinophiles in hydatid cysts following their complete surgical removal. Dr. Bronson Ray recently successfully removed in this hospital a cysticercus cyst of the brain from a missionary whose preoperative eosinophile level had been 11 per cent. This fell to 2 per cent six weeks after the operation.[69] Ashford[77] has noted that in the eosinophilia of uncinariasis, which varied from 6 per cent to 91 per cent in his 7 cases, the percentage rose when most of the worms in the intestine had been expelled. The eosinophilia in this disease rises with the larval mortality in the tissues and is not due to a toxin liberated by the worms but is at least related to the disintegration and absorption of foreign protein. Hampson,[29] commenting on the same phenomenon in echinococcus cysts, considers the eosinophilia a protective mechanism against small protein leaks. Even the phenomenon of reactions, especially the eosinophilia, in the nirvanol treatment of chorea[56] is probably on a foreign protein basis according to the most recent work of Landsteiner,[83] who has established the fact that simple chemical compounds can be converted into antigens by being attached to proteins.

There are certain experiments which tend to add a third factor, namely neural influence in the production of eosinophilia. Chillingworth, Healy, and Haskins[67] report a remarkable study done on 15 medical students. These men, whose average eosinophile count was 0.4 per cent before the experiment, were given a flutter valve with a partially blocked resistance, varying from 20 to 30 mm. of mercury, to blow into. Two and one-half minutes after the start of the exercise their eosinophile counts had risen to an average of 7.2 per cent. The explanation was offered that overdistention of the alveoli initiates reflex peripheral stimuli which bring about a release of eosinophiles from the blood-forming organs. In explanation of Banerji's experiments for producing eosinophilia in rabbits by increasing the carbon dioxide content of the blood, these authors believe that rather than the asphyxia being a specific stimulus it was the alveolar distention which resulted in a vagal stimulation. Its clinical counterpart, emphysema, with its elevated carbon dioxide tension, has no eosinophilia. If vagal stimulation is an important factor in the production of eosinophilia, we would expect to find definite effects from vagal stimulating drugs. This is accomplished most frequently by the use of digitalis preparations. Only two cases were found in the English literature.[63, 64] In neither of these cases were other parasympathetic stimulating drugs effective in raising the eosinophile count of the blood. In Geiger's case[64] the eosinophiles fell from 22 per cent to 2 per cent when digitalis was discontinued, and rose again to 22 per cent when it was resumed. Camp[68] obtained eosinophile rises in 82 per cent of his rabbits injected with parasympathetic stimulating drugs but also obtained increases in 65 per cent of other rabbits injected with sympathetic stimulating drugs. He concluded that eosinophilia was not produced by a specific neural stimulus.

The possibility of a hormonal influence on the eosinophile cell is suggested by several experimenters. Noguchi[72] and Port[73] were the first to note the increase in eosinophiles following splenectomy in man and experimental animals. It is apparently accepted as a definite phenomenon by most workers. These changes have already been mentioned in the cases reported in this paper. Mayr and Moncorps[5] found it to occur in guinea pigs forty to fifty days after splenectomy and lasting for about four months. These authors were able to reduce this eosinophilia by daily injections of protein-free splenic extract. Four daily doses in one guinea pig reduced the total eosinophiles from 1,400 per c.mm. to 200 per c.mm. Downs and Eddy[78] had previously found no effect on the eosinophiles in normal rabbits into which repeated injections of splenic extract were made. Waldbott[19] suggests that hypofunction of the spleen results in hypereosinophilia and hyperfunction of the spleen results in hypoeosinophilia.

The clinical application of this splenic extract has been followed by some interesting results. Mayr and Moncorps,[5] using 1 c.c. of a 1:1,000 protein-free splenic extract, obtained concomitant reductions in eosinophilia and pruritus in a case of scabies and in a case of arsenical dermatitis. In 42 cases of eczema their results were very satisfactory in that pruritus was rapidly abolished and the skin lesions cleared up. Waldbott[19] obtained "very satisfactory results" in 57 per cent of 51 cases of allergic asthma (their eosinophile

counts averaged 6.7 per cent) treated only with splenic extract. Von Zumbusch[79] and Paul[80] are extremely enthusiastic about the use of splenic extracts in the pruritic diseases. Chevallier[81, 82] was unable to obtain a relationship between relief from itching and fall in eosinophilia, but in 12 cases of pruritus from eczema or seborrheic dermatitis there was marked relief, even though the eosinophilia frequently was unaffected.

SUMMARY

It is apparent that the question of the mechanism of the production of eosinophilia is not a clear-cut one. However, from the work which has been reported and reviewed in this paper, it appears that certain theories have more merit than others.

If eosinophilia was the result merely of chemotaxis, there should be a quantitative relationship between the active agent and the eosinophilia. I have not been able to find this correlation in the cases of scarlet fever, pemphigus, and secondary syphilis, nor has anyone else, including Opie, whose work with differing numbers of trichinae has already been mentioned.

Except for Chillingworth's[67] experiments there is very little to be said for the vagal stimulation influence. Dr. Ray Holden[85] was able to produce "eosinophiles" at will in urine specimens by altering the pH before centrifuging the white blood cells down and staining them. He suggests this alteration in the pH of the blood, resulting from the induced hyperpnea, as an explanation for Chillingworth's experiments unless these cells were identified by supravital technique, a point which is not mentioned in the original paper. Both of the digitalis intoxications could be equally well explained on a drug sensitivity basis, such as probably occurs in nirvanol medication.

More work must be done on the spleen before its influence on eosinophilia can be definitely established. That there is some such factor seems clear, but until the true function of the spleen is elucidated this will remain problematical.

An allergic basis for eosinophilia seems most likely, the strongest points being the inability to reproduce it by a single injection of foreign protein, and the similar time relationship between the production of eosinophilia and the known production of allergy.

CONCLUSIONS

1. The function of the eosinophile is not definitely known.

2. There is no postfebrile rise in eosinophiles in the cases of typhoid fever reported in this study. The reduction is of possible diagnostic significance.

3. The eosinophilia in trichinosis and pemphigus has apparently no prognostic significance.

4. The production of eosinophilia experimentally favors most strongly an allergic basis.

It is a pleasure to express my indebtedness to Dr. Paul Reznikoff and to Dr. Eugene DuBois for their constructive criticism in the preparation of this paper.

REFERENCES

1. Ehrlich, P.: Methodologische Beiträge zur Physiologie und Pathologie der verschiedenen Formen des Leukocyten, Ztschr. f. klin. Med. **1**: 553, 1880.
2. Brown, T. R.: Studies in Trichinosis, Bull. Johns Hopkins Hosp. **73**: 79, 1897.
3. Thayer, W. S.: Discussion of Brown's paper.
4. Weidenrich, F.: Die Morphologie der Blutzellen und Ihre Beziehungen zu Einander, Anat. Rec. **4**: 317, 1910.
5. Mayr, J. K., and Moncorps, C.: Studien zur Eosinophilie, Virchows Arch. f. path. Anat. **264**: 774, 1927; **256**: 19, 1925.
6. Browning, C. H.: Observations on the Development of the Granular Leucocytes in the Human Foetus, J. Path. & Bact. **10**: 145, 1905.
7. Downey, H.: The Origin and Development of Eosinophilic Leucocytes and of Haematogenous Mast-Cells in the Bone Marrow of an Adult Guinea Pig, Folia. haemat. **19**: 148, 1915 (Quoted by C. H. Bunting).
8. Biggart, J. H.: Some Observations on the Eosinophile Cell, J. Path. & Bact. **35**: 799, 1932.
9. Bunting, C. H.: Special Cytology, New York, 1932, Paul B. Hoeber & Co., p. 693, E. V. Cowdry, Editor-in-Chief.
10. Von Noorden, C.: Beitrage zur Pathologie des Asthma Bronchiole, Ztschr. f. klin. Med. **20**: 98, 1892.
11. Cowie, D. M., and Jiminez, B.: Cytologic Examination of Nasal Smears of Sensitized and Non-sensitized Persons With Nasal Symptoms, Arch. Int. Med. **57**: 85, 1936.
12. Bayne-Jones, S.: Pleural Eosinophilia, Bull. Johns Hopkins Hosp. **27**: 12, 1916.
13. Baumgartner, E. A., and Cowles, A.: Trichinosis, With a Report of Two Cases With Eosinophilia in the Stools, J. Lab. & Clin. Med. **22**: 484, 1937.
14. O'Brien, C. S.: Ophthalmology, Iowa City, 1930, Athens Press, p. 67.
15. Bunting, C. H.: The Blood Picture in Hodgkin's Disease, Bull. Johns Hopkins Hosp. **22**: 369, 1911.
16. Kennan, B. R., Shipp, M. E., and Hetherington, D. C.: A Study of the White Blood Cell Picture in Six Young Men, Am. J. Physiol. **118**: 690, 1937.
17. Billings, J. S.: A Case of Asthma and Symmetrical Enlargement of the Arms, N. Y. Med. J. **65**: 691, 1897.
18. Herrick, W. W.: The Eosinophilia of Bronchial Asthma, J. A. M. A. **57**: 1837, 1911.
19. Waldbott, G. L.: Roentgen Ray Treatment of the Spleen in Asthma and in Related Allergic Diseases, Arch. Int. Med. **41**: 683, 1928.
20. Zappert, J.: Ueber das Vorkommen der Eosinophilen Zellen im Menschlichen Blute, Ztschr. f. klin. Med. **23**: 227, 1892.
21. Friedman, G., and Holz, E.: The Behavior of the Eosinophiles in Rheumatic Fever, J. Lab. & Clin. Med. **21**: 225, 1935.
22. Friedman, S.: Eosinophilia in Scarlet Fever, Am. J. Dis. Child. **49**: 993 and 1256, 1935.
23. Bücklers: Ueber den Zusammenhang der Vermehrung der Eosinophilen Zellen im Blute mit dem Vorkommen der Charcotschen Krystalle in den Fäces bei Wurmkranken, München. med. Wchnschr., January 29, 1894, p. 21.
24. Müller, H. F., and Rieder, H.: Ueber Vorkommen und Klinische Bedeutung der Eosinophilen Zellen (Ehrlich) im Circulirenden Blute des Menschen, Deutsches Arch. f. klin. Med. **48**: 96, 1891.
25. Calvert, W. J.: Eosinophilia in Filariasis, Bull. Johns Hopkins Hosp. **13**: 133, 1902.
26. Page, I. H., Turner, K. B., and Wilson, J. H.: The Clinical Significance of Eosinophilia on a General Medical Service, J. Lab. & Clin. Med. **13**: 1109, 1928.
27. Coles, A. C.: The Blood in Cases Affected With Filariasis and Bilharzia Hematoba, Brit. M. J. **1**: 1137, 1902.
28. Hinman, E. H.: A Study of 85 Cases of Strongyloides Stercoralis Infection With Special Reference to Abdominal Pain, Tr. Roy. Soc. Trop. Med. & Hyg. **30**: 531, 1937.
29. Hampson, A. C.: Eosinophilia in Echinococcus Infection, Guy's Hosp. Rep. **75**: 313, 1925.
30. Quoted by Lamb, A. R.: Periarteritis Nodosa, a Clinical and Pathological Review of the Disease, Arch. Int. Med. **14**: 481, 1914.
31. Middleton, W. S., and McCarter, J. C.: The Diagnosis of Periarteritis Nodosa, Am. J. M. Sc. **190**: 291, 1935.
32. Schamberg, J. F., and Strickler, A.: Report of Eosinophilia in Scabies With a Discussion of Eosinophilia in Various Diseases of the Skin, J. Cutan. Dis. **30**: 53, 1912.
33. Bezancon, F., and Moreau, R.: L'Eosinophilie dans les diathèses et les états anaphylactiques, Ann. de méd. **2**: 85, 1914.
34. Bittorf, A.: Eosinophilie des Blutes bei Muskelrheumatismus, Deutsche med. Wchnschr. **45**: 354, 1919.

35. Stillman, R. G.: Myeloid Leukemia With Preponderance of Eosinophile Cells, Med. Rec. **81**: 594, 1912.
36. Hay, J., and Evans, W. H.: Acute Eosinophilic Leukemia and Eosinophilic Erythroleukemia, Quart. J. Med. **22**: 167, 1929.
37. McDonald, S., and Shaw, A. F. B.: Persistent Eosinophilia With Splenomegaly, Brit. M. J. **2**: 966, 1922.
38. Harrison, F. H.: Eosinophilia With Splenomegaly, Am. J. M. Sc. **179**: 208, 1930.
39. Giffin, H. Z.: Persistent Eosinophilia With Hyperleucocytosis and Splenomegaly, Am. J. M. Sc. **158**: 618, 1919.
40. Drennan, A. M., and Biggart, J. H.: Persistent Eosinophilia, J. Path. & Bact. **33**: 995, 1930.
41. Stephens, D. J.: Acute Eosinophilic Leukemia, Am. J. M. Sc. **189**: 387, 1935.
42. Krohn, S. E.: Acute Recurrent Polymyositis Associated With Blood Eosinophilia, New York State J. Med. **37**: 10, 1937.
43. Quoted by Hansson, Nils: Transitory Lung Infiltration With Eosinophilia, Acta radiol. **18**: 207, 1937.
44. Spangler, R. H.: Eosinophilia in Syphilis, J. Lab. & Clin. Med. **20**: 733, 1935.
45. Kanthack, A. A.: The Diagnostic Value of the Eosinophile Leucocytes in Leukemia and Hodgkin's Disease, Brit. M. J. **2**: 120, 1892.
46. Weber, F. Parkes: Abdominal Lymphogranulomatosis Maligna (Hodgkin's Disease) With Very High Blood Eosinophilia, Proc. Roy. Soc. Med. **20**: 1337, 1927.
47. Sternberg, L.: Eosinophilia in Hay Fever, Am. J. M. Sc. **176**: 82, 1928.
48. Warfield, L. M., and Kristjanson, H. T.: Hodgkin's Disease of the Intestines, Bull. Johns Hopkins Hosp. **27**: 24, 1916.
49. Klinkert, D.: Ueber familiare (erbliche) Eosinophilie, Berl. klin. Wchnschr. **48**: 938, 1911.
50. Armand-Delille, P. F., Hurst, A. F., and Sorapure, V. E.: Familial Eosinophilia, Guy's Hosp. Rep. **80**: 248, 1930.
51. Brannan, D.: Chloroma, Bull. Johns Hopkins Hosp. **38**: 189, 1926.
52. Opie, E. L.: The Occurrence of Cells With Eosinophile Granulation and Their Relation to Nutrition, Am. J. M. Sc. **127**: 217, 1904.
53. Rous, F. P.: Some Differential Counts of the Cells in the Lymph of the Dog, J. Exper. Med. **10**: 537, 1908.
54. Campbell, A. C., Drennan, A. M., and Rettie, T.: The Relationship of the Eosinophile Leucocyte to Allergy and Anaphylaxis, J. Path. & Bact. **40**: 537, 1935.
55. Vaughn, S.: The Leukocyte Response in Man to Dick Toxin, With Special Reference to Eosinophile Changes, J. Infect. Dis. **50**: 315, 1932.
56. Whitaker, W. M.: The Nirvanol Treatment of Chorea, Arch. Dis. Childhood **5**: 44, 1930.
57. Tisdall, O. R.: The Nirvanol Treatment of Chorea, Ibid. **5**: 396, 1930.
58. Weinberg, M., and Séguin, P.: Réchèrches Biologiques sur L'Eosinophile, Deuxieme Partie, Ann. Inst. Pasteur **29**: 323, 1915.
59. Opie, E. L.: An Experimental Study of the Relation of Cells With Eosinophile Granulation to Infection With an Animal Parasite (Trichina Spiralis), Am. J. M. Sc. **127**: 477, 1904.
60. Herrick, W. W.: Experimental Eosinophilia With an Extract of an Animal Parasite, Arch. Int. Med. **11**: 167, 1913.
61. Meulengracht, E., and Holm, S.: Eosinophilia in Liver Diet, Am. J. M. Sc. **179**: 199, 1930.
62. Lawrence, R. D., and Buckley, O. B.: Eosinophilia in Insulin Therapy, Brit. M. J. **1**: 597, 1929.
63. Smith, A. E., and Benner, S. R.: Eosinophilia Due to the Administration of Digitalis, Am. Heart J. **7**: 182, 1931.
64. Romano, J., and Geiger, A.: Digitalis Eosinophilia, Am. Heart J. **11**: 742, 1936.
65. Archard and Lepper: Quoted by Sternberg.[47]
66. Banerji, N.: The Induction of Eosinophilia in Normal Animals, Am. J. M. Sc. **186**: 689, 1933.
67. Chillingworth, F. P., Healy, J. C., and Haskins, F. E.: Reflex Eosinophilia, J. Lab. & Clin. Med. **19**: 486, 1934.
68. Camp, W. J. R.: The Effect of Drugs on the Number of Circulating White Blood Cells, J. Lab. & Clin. Med. **13**: 206, 1927.
69. Ray, Bronson. To be published.
70. Most, H., and Abeles, M. M.: Trichiniasis Involving the Nervous System, Arch. Neurol. & Psychiat. **37**: 589, 1937.

71. Reiche, M.: Med. Klin. **14**: 984, 1918; Quoted by Silver, S.: Arch. Int. Med. **51**: 175, 1933.
72. Noguchi, Y.: Ueber die Extirpation der normalen Milz beim Menschen, Berl. klin. Wchnschr. **49**: 1839, 1912.
73. Port, F.: Die Bedeutung der milz als hämatopoetisches Organ. Zugleich ein Beitrag zum normalen Blutbild des Kaninschens, Arch. f. exper. Path. u. Pharmakol. **73**: 251, 1913.
74. Seeman, G., and Sajzewa, A.: Ein fall von Chloromyelose mit ungewohnlicher Eosinophilie, Folia haemat. **37**: 258, 1928.
75. Grace, A. W.: Pemphigus, Arch. Dermat. & Syph. **30**: 22, 1934.
76. Foot, N. C.: The Identification of Tumor Cells in Sediments of Serous Effusions, Am. J. Path. **13**: 1, 1937.
77. Ashford, B. K., Payne, G. C., and Payne, F. K.: Acute Uncinariasis From Massive Infestation and Its Implication, J. A. M. A. **101**: 843, 1933.
78. Downs, A. W., and Eddy, N. B.: The Influence of Splenic Extract on the Number of Corpuscles in the Circulating Blood, Am. J. Physiol. **51**: 279, 1920.
79. von Zumbusch, Leo.: The Question of Eczema and the Exudative Diathesis, Urol. & Cutan. Rev. **31**: 750, 1927.
80. Paul, T. M.: The Instantaneous Physiologic Cure of Eczema and Urticaria, Urol. & Cutan. Rev. **33**: 724, 1929. See also Ibid. **34**: 380, 1930.
81. Chevallier, Paul, and Bloch, Leon: L'extrait aqueux de rate dans le traitement des prurits et des eczemas, Bull. et mém. Soc. méd. d. hôp. de Paris **54**: 1814, 1930.
82. Chevallier, Paul, and Ely, Z.: Action de l'extrait aqueux de rate sur l'eosinophilie sanguine, Ibid. **54**: 1819, 1930.
83. Landsteiner, Karl: Serological and Allergic Reactions With Simple Chemical Compounds, New England J. Med. **215**: 1199, 1936.
84. Battaglio: Cause of Eosinophilia in Tumors, Tumori **13**: 191, 1927. (Quoted by Biggart.[8])
85. Holden, R. F.: Personal communication.

CHANGES IN THE COLLOIDAL GOLD CURVE OF NORMAL AND PATHOLOGIC SPINAL FLUIDS AFTER ULTRAVIOLET IRRADIATION*

JOEL WARREN, M.A., NEW YORK, N. Y.

THE specific agents concerned in the precipitation of a solution of colloidal gold by certain pathologic spinal fluids remain, twenty-five years after Lange's discovery, a matter of controversy. The assumption that the mechanism is one of neutralization of negative charges carried by finely dispersed colloid particles with a subsequent precipitation of the neutral aggregate is agreed to by most workers (Zsigmondy, Jaeger, Goldstein, Mellanby and Anwyl-Davies). When one of the colloid components of the system is in excess, a protective action is observed both against spontaneous and electrolytic flocculation. This phenomenon was exhaustively studied by Zsigmondy who measured the protective action of certain colloids, notably proteins, on the precipitation of gold sols, and found the degree of protection to be specific for each protein studied.

*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.
Received for publication, January 7, 1938.

Most workers still regard proteins as the agents in abnormal spinal fluids responsible for the precipitation of colloidal gold. Weston, Mellanby and Anwyl-Davies believed the essential factor to be a globulin, since precipitation did not occur after removal of the globulin by ammonium sulfate. Cruickshank was able to simulate pathologic gold curves with suitable mixtures of albumin and globulin. Mellanby and Anwyl-Davies confirmed this. It seems, therefore, that proteins are the agents, alone or with inorganic radicals, which cause characteristic gold precipitation curves in abnormal spinal fluids. Our work, while not furnishing absolute confirmation of this hypothesis, has yielded some highly suggestive results when considered in conjunction with the findings of other workers.

METHODS

Normal and abnormal spinal fluids were secured from the routine diagnostic services of the Vanderbilt Clinic and Presbyterian Hospital. Ten cubic centimeter amounts of fluid in rock crystal vessels were exposed for various lengths of time to a mercury vapor ultraviolet lamp (Lepel) at about 25 cm. distance. The spectrum was polychromatic, waves from 2,800 to 3,100 Å predominating. These wave lengths are almost completely absorbed by the spinal fluid proteins (Skinner). The temperature variations during irradiation were not of significance. Determination of pH values (colorimetric) were made after irradiation.

The colloidal gold test was run before and after raying in the usual manner, all due precautions being taken in the preparation of the reagents, vessels, etc. Final dilutions of spinal fluid were from 1:35 to 1:17920.

RESULTS

Normal Spinal Fluid.—Eight normal spinal fluids were rayed for various intervals, i.e., from five minutes to sixty minutes.

SPINAL FLUID NO.	TIME RAYED	CURVE BEFORE RAYING	CURVE AFTER RAYING	pH AFTER RAYING
1	5 min.	1111100000	1111100000	7.5
2	5 min.	1111000000	1111000000	7.4
3	10 min.	1111100000	1121100000	7.4
4	15 min.	1111100000	1111100000	7.5
5	17 min.	1111100000	1111100000	7.3
6	30 min.	1111100000	1111100000	7.5
7	60 min.	1111000000	1111100000	7.4

No significant changes were noted in the curves of these normal fluids.

Spinal Fluids With Paretic Curves.—Four paretic spinal fluids were rayed for periods of time, varying from five to sixty minutes.

SPINAL FLUID NO.	TIME RAYED	CURVE BEFORE RAYING	CURVE AFTER RAYING	pH AFTER RAYING
8	5 min.	5543211000	5211000000	7.5
9	5 min.	5554321100	5421000000	7.7
10	30 min.	5444321100	4322100000	7.4
11	60 min.	5543211000	5211000000	7.5

It will be seen that all four fluids underwent similar types of change, i.e., they exhibited a tendency for decreased precipitation in the highest concentrations. Thus dilutions between 1:70 and 1:1220 showed a decrease in gold precipitation after only five minutes of raying. However, the strongest dilution of spinal fluid, or 1:35, remained unaffected even after sixty minutes of irradiation. It is possible that raying for longer than sixty minutes might decrease the amount of precipitation in tube No. 1.

Spinal Fluids With Syphilitic Curves.—Four spinal fluids giving syphilitic curves were rayed for varying intervals:

SPINAL FLUID	TIME RAYED	CURVE BEFORE RAYING	CURVE AFTER RAYING	pH AFTER RAYING
12	5 min.	1232111000	3211000000	7.3
13	15 min.	1232111000	3211100000	7.4
14	30 min.	1233211000	2111100000	7.4
15	60 min.	1232110000	3211100000	7.4

These fluids after exposure to ultraviolet light showed an increased precipitation in the region of highest concentration, beginning with the first tube of the series. Variations in the length of exposure did not seem to be of great significance within the limits tried. It will be noted that these results are the opposite of what occurred when paretic fluids were rayed.

DISCUSSION

Explanation of this phenomenon requires some discussion of the flocculation of colloidal gold sols by proteins and the effects of ultraviolet light upon these proteins. Reznikoff made a study of the action of euglobulin, pseudoglobulin, and albumin on the colloidal gold curve. Mixtures of the three proteins gave colloidal gold curves which depended on the relative concentration of these constituents. Similarly, Kafka and Samson declared that the discharge of colloidal gold is roughly related to the albumin-globulin ratio, as well as to the total protein content of the spinal fluid. They concluded that the active agent in precipitation is globulin, whereas albumin possesses a protective action.

It is of interest now to determine whether ultraviolet irradiation is capable of changing the albumin-globulin composition of spinal fluid.

Even if the nature of such changes might not be as clear as one would desire, sufficient information is available to offer a working hypothesis. Clark reported that serum albumin at pH 7.4 carries a negative charge which upon irradiation with a quartz mercury lamp becomes positive. Globulin, on the other hand, according to Mond, undergoes no change in the nature of the electrical charge and its stability increased after irradiation.

It will be noted that irradiation of normal spinal fluid produced no change in the type of curve. Normal spinal fluid is low in total protein, particularly so in globulin (Solomon). Since globulin appears to be the precipitating agent of colloidal gold, and ultraviolet irradiation merely increases the stability of globulins, we should, therefore, not expect any important changes in the curve

of normal spinal fluid. The effect of irradiation on albumin can be neglected, since the latter is present only in traces in normal spinal fluid.

Changes produced by irradiation in the colloidal gold curves of spinal fluid of the paretic type may now be discussed on the same basis. Such fluids show ordinarily an average increase in total protein of over 100 per cent (Merritt and Fremont-Smith), due to an increase in globulins and albumin, with the latter always exceeding the former (Solomon, p. 407, Greenfield and Carmichael). This is what actually takes place inasmuch as treatment of paretic fluids with ultraviolet light tends to decrease the precipitation in the first dilutions. Since irradiation produces two fundamental changes, i.e., reversal of charge of the serum albumin, as well as an increase in stability of serum globulin, one might expect that the precipitating effect of the albumin is counteracted by a stabilizing effect of the less dissociated globulin, resulting in a lesser precipitation of the colloidal gold.

The most striking changes induced by irradiation were in spinal fluids with syphilitic gold curves. After treatment such fluids reverted essentially to paretic curves with precipitation at the beginning rather than in the middle of the curve. This was to be expected as the prezone in the unrayed fluid is due to a protective action of albumin. After raying, the albumin molecule loses its negative charge and its antagonism to globulin disappears. Therefore, precipitation would tend to occur in the first few tubes and decrease in the middle tubes, coincident with a lesser dissociation of the globulin.

It is open to question whether this adequately explains all of the mechanism of ultraviolet irradiation on the colloidal gold curve of spinal fluids. That proteins are the essential agents in this reaction is practically certain. However, since ultraviolet light produces the changes in serum globulin and albumin described above, and since the behavior of the colloidal gold curve after irradiation is in accordance with the assumption of antagonistic albumin-globulin ratios, we feel that this explanation is in harmony with the observed facts.

SUMMARY

Irradiation of normal spinal fluids with ultraviolet light produces no significant change in the colloidal gold reaction of such fluids.

When spinal fluids giving paretic curves are irradiated, the amount of gold precipitated is decreased in the highest concentrations.

Fluids with syphilitic colloidal gold curves, after irradiation, show a decreased precipitation in the middle dilutions and increased precipitations in the highest concentrations.

The variations produced by changes in the protein composition of spinal fluids are discussed. The effect of ultraviolet light on these proteins is offered as an explanation of the modifications of the colloidal gold curve after irradiation of the spinal fluid.

REFERENCES

Clark, J. H.: A Simple Method of Measuring Intensity of Ultraviolet Light With Comparable Results on a Number of Physiological Reactions, Am. J. Physiol. **69**: 200, 1924.

Clark, J. H.: Studies on Radiated Proteins; Coagulation of Egg Albumin by Ultraviolet Light and Heat, Am. J. Physiol. **73**: 649, 1925.
Cruickshank, J.: The Value and Mechanism of the Colloidal Gold Test, Brit. J. Exper. Path. **1**: 71, 1920.
Greenfield, J. G., and Carmichael, E. A.: The Human Cerebrospinal Fluid, London, 1925, The Macmillan Co.
Jaeger, K., and Goldstein, P.: Goldsolreaktion im Liquor Cerebrospinalis, Ztschr. f. d. ges. Neurol. u. Psychiat. **26**: 318, 1914.
Kafka, V.: Die Zerebrospinalflüssigkeit, Leipzig, 1930, Franz Deuticke.
Lange, C.: Die Ausflockung kolloidalen Goldes durch Cerebrospinalflussigkeit bei luetischen Affecktionen des Zentralnervensystems, Ztschr. f. Chemotherap. **1**: 44, 1912.
Mellanby, J., and Anwyl-Davies, T.: The Precipitation of Colloidal Gold by Cerebrospinal Fluid; the Diagnosis of Neurosyphilis, Brit. J. Exper. Path. **4**: 132, 1923.
Merritt, H. H., and Fremont-Smith, F.: The Cerebrospinal Fluid, Philadelphia, 1937, W. B. Saunders Co.
Mond, R.: Untersuchungen über die Wirkung der ultravioletten Strahlen auf Eiweislosungen, 1 Mitteilung, Pflüger's Arch. f. d. ges. Physiol. **196**: 540, 1922.
Mond, R.: Untersuchungen über die Wirkung der ultravioletten Strahlen auf Eiweislosungen, 11 Mitteilung, Pflüger's Arch. f. d. ges. Physiol. **200**: 374, 1923.
Reznikoff, P.: The Action of Proteins of Blood Serum on Colloidal Gold Solution and Its Quantitative Interpretation, J. Lab. & Clin. Med. **8**: 92, 1923.
Samson, K.: Physikalische-chemische Grundlagen der Mastix-reaktion; das Verhalten der Liquoreiweiskorper bei der Normo Mastix-reaktion, Ztschr. f. d. ges. exper. Med. **60**: 665, 1925.
Skinner, E. F.: An Investigation of the Absorption of Ultraviolet Light by Cerebrospinal Fluid in Various Disease States, J. Neurol. & Psychopath. **17**: 213, 1937.
Solomon, H. C.: The Human Cerebrospinal Fluid, Association for Research in Nervous and Mental Diseases, New York, 1926, Paul B. Hoeber.
Spiegal-Adolph, M.: Physikalische-chemische Untersuchungen bestrahlter Proteine; die Veranderungen des Seralbumins bei Ultraviolettbestrahlung und ihre Beziehungen zur Hitzegerinnung, Biochem. Ztschr. **186**: 181, 1927.
Zsigmondy, R.: Zur erkenntnis der Kolloide; über irreversible hydrosole und ultramikroskopie, Jena, 1905, Gustav Fischer.

THE PRESENT STATUS OF THE STAPHYLOCOCCUS FOOD POISONING PROBLEM*

Thomas C. Grubb, Ph.D., Baltimore, Md.

FOLLOWING the discovery by Dack and his co-workers[1] in 1930 that certain strains of staphylococci produce a gastrointestinal irritant (enterotoxin) which causes food poisoning, many reports of staphylococcus food poisoning outbreaks have been published (summarized by Kelly and Dack[2]). In establishing the etiologic relationship of staphylococci isolated from the incriminated food as the cause of the outbreak, it is customary to feed filtrates of the organisms to human volunteers or monkeys (*M. rhesus*). Because the feeding of human volunteers causes considerable unpleasantness and some danger if a positive result is obtained, and the feeding of monkeys is frequently impracticable, attempts have been made to develop an in vitro test for distinguishing food poisoning staphylococci. In seeking such a test, however, several investigators have failed to take cognizance of some of the fundamental factors in the problem, thereby leading to an increasing amount of confusing information in the literature. It is for the purpose of helping to clarify this confusion so that

*From the Department of Bacteriology, University of Maryland School of Pharmacy.
Received for publication, January 8, 1938.

previous work may be properly evaluated and subsequent investigations prove more fruitful, that this report is submitted.

Although the original experimental work establishing the rôle of staphylococci in food poisoning was based to a large extent on the production of vomiting in monkeys fed with toxic filtrates, it is now realized that monkey feeding experiments are not entirely reliable, because Dack[3] and Shaughnessy and Grubb[4] have found that these animals cannot be relied upon to vomit consistently when a toxic filtrate is fed. Chinn[5] states that he has observed monkeys vomit after being fed sterile nutrient broth. Therefore, monkey feeding experiments alone are not reliable in determining the enterotoxin production of staphylococci. In the light of this fact, previous work, where conclusions were based entirely upon the results of monkey feeding experiments, should be reconsidered.

In 1935, Stone[6] reported that food poisoning strains of staphylococci liquefied his specially prepared gelatin medium. Chinn[7] was unable to verify Stone's conclusions. Since Chinn did not carry out concomitant animal or human feeding experiments while testing Stone's medium, his conclusions cannot be considered valid, because it is known that strains originally isolated from food poisoning sources may lose their ability to produce an enterotoxin after cultivation on artificial media.[8] Doleman and his co-workers,[9] Gwatkin[10] and Kupchik,[11] all of whom did concomitant animal tests, were unable to confirm Stone's findings.

When an investigator attempts to find an in vitro test for distinguishing food poisoning staphylococci by taking one group of strains, isolated from food poisoning outbreaks, and another group of strains from other sources (air, water, pus, throat, etc.) and compares their reactions on a given medium without concomitant animal or human feeding experiments, his conclusions cannot be considered valid, because it is known that strains from food poisoning sources may lose their enterotoxigenic power,[8] and strains from nonfood-poisoning sources are frequently capable of producing an enterotoxin.[12] Therefore, to obtain unequivocal results in any experiment of this type, it must be shown by concomitant feeding experiments that the strains being tested do or do not produce an enterotoxin, *regardless of their original source*. The reports of Hucker and Haynes[13] and Chapman and associates[14] should be reconsidered with these facts in mind. At the present time, the feeding of human volunteers offers the most reliable means of testing a filtrate, although even this method is beset with difficulties since Kelly and Dack[2] and Dennison[15] have found that some persons are entirely unaffected by staphylococcus enterotoxin. However, the recently developed kitten injection method of Doleman,[9] successfully employed by Kupchik,[11] appears to be a promising substitute.

Chapman and his co-workers[16-18] have published a series of papers in which they report the correlation of certain in vitro reactions of staphylococci with the "pathogenicity" of the strains. It may be noted in passing that since their criteria for pathogenicity depend solely upon the ability of the strain to kill rabbits after an intravenous injection, these in vitro tests alone should not yet be

used to identify human pathogenic strains, for, as Julianelle[19] has remarked, a strain which is pathogenic for animals is not always pathogenic for human beings, and vice versa. Chapman and his co-workers[14] have recently applied these same in vitro tests to staphylococci from food poisoning and nonfood-poisoning sources in an attempt to identify food poisoning strains. Their conclusions should not be accepted without considerable reservation, because they did not run concomitant feeding experiments on all strains tested; and they assumed, in the face of considerable evidence to the contrary,[15, 20, 21] that the nonhemolytic strains they encountered were by virtue of their lack of hemotoxin, not the original food poisoning strains, but "degenerate" variants or contaminants.

Finally a warning should be sounded, that, as Topley and Wilson[22] very pointedly remark, the mere demonstration that bacteria isolated from foods implicated in food poisoning outbreaks are capable of producing a toxin, is not incontrovertible evidence that these bacteria caused the outbreak. This warning is particularly apropos in the case of staphylococci, which being ubiquitous, makes it especially difficult to incriminate them in a food poisoning outbreak, unless it can be shown beyond reasonable doubt that they did not gain access to, or multiply in, the suspected food sample during the process of obtaining the sample and transporting it to the laboratory. It must further be remembered that even though staphylococci, shown to produce an enterotoxin by suitable laboratory manipulations and feeding experiments, are isolated from the incriminated food, these organisms may not have produced an enterotoxin in the food, due to absence of certain necessary conditions of pH, carbon dioxide tension, and temperature.

REFERENCES

1. Dack, G. M., Cary, W. E., Woolpert, O. C., and Wiggers, H.: An Outbreak of Food Poisoning Proved to Be Due to a Yellow Hemolytic Staphylococcus, J. Prev. Med. **4**: 167, 1930.
2. Kelly, F. C., and Dack, G. M.: Experimental Staphylococcus Food Poisoning, Am. J. Pub. Health **26**: 1077, 1936.
3. Dack, G. M.: Personal communication.
4. Shaughnessy, H. J., and Grubb, T. C.: The Incrimination of Milk and Milk Products in Staphylococcus Poisonings, Canad. Pub. Health J. **28**: 229, 1937.
5. Chinn, B. D.: Personal communication.
6. Stone, R. V.: A Cultural Method for Classifying Staphylococci as of the "Food Poisoning" Type, Proc. Soc. Exper. Biol. & Med. **33**: 185, 1935.
7. Chinn, B. D.: Observations on Reactions of Staphylococci of the Food Poisoning Types in Gelatin, Food Research **1**: 513, 1936.
8. Jordan, E. O., and Burrows, W.: The Production of Enterotoxic Substance by Bacteria, J. Infect. Dis. **57**: 121, 1935.
9. Doleman, C. E., Wilson, R. J., and Cockcroft, W. H.: A New Method of Detecting Staphylococcus Enterotoxin, Canad. Pub. Health J. **27**: 489, 1936.
10. Gwatkin, R.: Further Observations on Staphylococcus Infections of the Bovine Udder, Canad. Pub. Health J. **28**: 185, 1937.
11. Kupchik, G. J.: Some Cultural and Biochemical Characteristics of Enterotoxic Staphylococci, J. Infect. Dis. **61**: 320, 1937.
12. Jordan, E. O.: Staphylococcus Food Poisoning, J. A. M. A. **97**: 1704, 1931.
13. Hucker, G. J., and Haynes, W. C.: Certain Factors Affecting the Growth of Food Poisoning Micrococci, Am. J. Pub. Health **27**: 590, 1937.

14. Chapman, G. H., Lieb, C. W., and Curcio, L. G.: Isolation and Cultural Differentiation of Food Poisoning Staphylococci, Food Research **2**: 349, 1937.

15. Dennison, G. A.: Epidemiology and Symptomatology of Staphylococcus Food Poisoning, Am. J. Pub. Health **26**: 1168, 1936.

16. Chapman, G. H., Berens, C., Peters, A., and Curcio, L.: Coagulase and Hemolysin Tests as Measures of the Pathogenicity of Staphylococci, J. Bact. **28**: 343, 1934.

17. Chapman, G. H., and Berens, C.: Crystal Violet as a Differential Medium for Staphylococci, J. Bact. **39**: 437, 1935.

18. Chapman, G. H., Lieb, C. W., Berens, C., and Curcio, L.: The Isolation of Probable Pathogenic Staphylococci, J. Bact. **33**: 533, 1937.

19. Julianelle, L. A.: Determination of Staphylococcal Types by Fermentation of Mannite, Proc. Soc. Exper. Biol. & Med. **36**: 617, 1937.

20. Woolpert, O. C., and Dack, G. M.: Relation of Gastro-Intestinal Poison to Other Toxic Substances Produced by Staphylococci, J. Infect. Dis. **52**: 6, 1933.

21. Stritar, J., and Jordan, E. O.: Is a Special Type of Staphylococcus Concerned in Food Poisoning? J. Infect. Dis. **56**: 1, 1935.

22. Topley, W. W. C., and Wilson, G. S.: The Principles of Bacteriology and Immunity, ed. 2, Baltimore, 1936, William Wood & Co., p. 1268.

THE EFFECT UPON SODIUM CHLORIDE CRYSTAL GROWTH OF CONTAMINATION WITH NORMAL AND ABNORMAL CEREBROSPINAL FLUIDS*

ISIDORE FINKELMAN, M.S., M.D., CHICAGO, ILL.

THE growth of crystals from a salt solution in which the solvent is evaporated follows a definite physical sequence, and the resulting crystal configuration is peculiar to each salt. This is known as the crystal habit of a salt. All crystals contain lines called axes, and the surfaces and faces are grouped around the axes in definite positions. The axes connect angles, edges, or faces which are similarly situated on opposite sides of the crystal. For example, a cube has three equal axes at right angles to one another and terminating in the center of each of the six bounding surfaces. Sodium chloride crystals, when crystallized out of a solution, have a cubical shape, but the presence of impurities in the solution alters crystal habit, causing arborization or the formation of needlelike or dendritic crystals.

Pfeiffer[1] studied the changes produced in the crystallization of copper chloride from its solution by the addition of minute amounts of plant or animal extracts and diluted blood from patients with various diseases. He was able to differentiate seedlings of diseased from healthy trees and reported crystal patterns peculiar to diseases, such as tuberculosis and cancer. Trumpp and Rascher,[2] following Pfeiffer's technique, observed critical changes produced in the crystallization of copper chloride by the addition of small amounts of hormones, such as thyroxin, adrenalin, and pituitrin. The crystal picture was

*From the Department of Nervous and Mental Diseases, Northwestern University Medical School, and the Elgin State Hospital.

Read and exhibited at the Annual Meeting of the Central Neuropsychiatric Association, Chicago, October 9, 1937.

Received for publication, January 8, 1938.

peculiar to each hormone. A distinctive crystal pattern was produced by urine from pregnant women, differing from the pattern prepared with urine from nonpregnant women.

The crystals found after the evaporation of cerebrospinal fluid itself have not been studied very extensively. Mott[3] described cholin crystals in the fluid of patients suffering from disease producing a degeneration of nerve tissue. Thompson[4] reported the presence of elongated, colorless, transparent crystals in septic meningitis, and thought the radical present in the crystal was phosphoric acid. Levinson[5] was the first to make a systematic study of the crystallization process in a great number of pathologic fluids which were allowed to evaporate at room temperature. He found that after several months of standing most pathologic fluids showed the presence of many crystals of various sizes and shapes different from those found in normal fluids. Henning and Beck[6] noted differences in the evaporated drops of normal and pathologic cerebrospinal fluid. The pathologic picture showed a circumferential zone composed of albumin, and there were changes in the central crystalline structure. Keller and Schoen[7] also found differences in the fluorescent properties of the ring zone and the crystals in the dried drops of normal and pathologic cerebrospinal fluid. Zeiner-Henriksen[8] reported diagnostic significance in the crystal formation of spinal fluid in dementia paralytica. All of these authors, however, studied the crystals found in cerebrospinal fluid and not the effect on the crystallization of a salt from its solution by the addition of cerebrospinal fluid as a contaminant.

Without mentioning the work of Pfeiffer on the effect on the crystallization of copper chloride of small amounts of human blood, Tomesco, Cosmulesco, and Serban[9] reported changes in the crystallization of sodium chloride produced by the addition of a drop of cerebrospinal fluid to various amounts of 0.8 per cent saline. They found that when a drop of 0.8 per cent sodium chloride is evaporated at 75° C., there results, at the site of the drop, a crystalline circumference surrounding an empty space. If to the saline solution normal cerebrospinal fluid is added in the proportion of 1 drop to 1 c.c., 1 drop to 2 c.c., 1 drop to 3 c.c., etc., and a drop of the mixture is placed on a glass slide and evaporated as above, the area surrounded by the crystalline circumference is occupied by a translucent, crystalline layer which, with greater dilutions, begins to show empty spaces. In dilutions of 1 drop to 5 c.c. and 1 drop to 6 c.c., this layer is reduced to a very narrow strip along the marginal circumference.

On microscopic examination it is observed that this translucent area consists of rosettes, concentric circles, parallel lines, and crystalline needles. The pattern, especially in higher dilutions, is poorly organized, and the individual lines of the rosettes, concentric circles, or parallel lines appear as if drawn by a blunt thick pen. This is described as the normal type.

The pathologic type differs from the normal in that with dilutions from 1 drop to 1 c.c. to 1 drop to 6 c.c. the whole surface is covered by the crystalline, translucent layer. Macroscopically, these are characterized by the appearance of empty spaces in the central translucent layer at dilutions greater than with

the normal type (1 drop to 4 c.c. to 1 drop to 6 c.c.). Microscopically, rosettes, concentric circles, and parallel lines are observed, but their patterns are of great fineness as though drawn with a very fine pen.

There are various gradations between the normal and pathologic types which Tomesco and his co-workers term "mixed." Tomesco and others found that cerebrospinal fluid from patients with dementia paralytica furnished consistent pathologic patterns of sodium chloride crystallization. There was a correlation between the intensity of the albumin and globulin reactions of the spinal fluid and the degree of abnormality of the crystallization patterns, but the parallelism was not perfect.

METHOD

The technique outlined by Tomesco and associates was carefully followed. To each of a series of tubes containing 1, 2, 3, 4, 5, 6, etc., up to 10 c.c. of 0.8 per cent sodium chloride, 1 drop of spinal fluid was added, and each tube was shaken thoroughly. Four or five drops from each tube were placed on a glass slide, using a different slide and different pipettes for each dilution. The slides were then placed in an oven at 75° C. and kept there until the drops were completely evaporated. It was found practical as the work progressed to use only tubes containing 4, 5, 6, and 10 c.c., as such a series gives one complete information. Great care is taken in cleaning the slides with alcohol and ether.

NORMAL AND PATHOLOGIC CRYSTAL PICTURES

Observations were made of the changes in crystal growth caused by the contamination of 0.8 per cent sodium chloride solution with cerebrospinal fluid from patients with dementia paralytica, functional epilepsy, dementia praecox, various neuropsychiatric disorders, and nonneurologic disorders. The findings are in entire agreement with those of Tomesco and co-workers.

A drop of 0.8 per cent sodium chloride solution, without the addition of spinal fluid as an impurity, after evaporation, shows a crystalline circumference surrounding an empty space where the drop had been. Microscopically this consists of three zones—a middle zone of large, slender, pale, coalesced crystals which is continuous with an inner zone of an unformed gray mass of crystals and a similar outer zone.

The addition of 1 drop of normal cerebrospinal fluid shows open spaces in the translucent crystalline layer at a dilution of 1 drop to 4 c.c. and completely open spaces, excepting a marginal strip at dilutions of 1 drop to 5 c.c., 1 drop to 6 c.c., and higher dilutions (Fig. 1). The translucent layer is also less dense in the normal picture than in pathologic preparations at corresponding dilutions. Microscopically, the circumference consists of only one zone of cubical sodium chloride crystals which are dentated toward the center and amputated at the periphery where they form the circle of the original drop. The translucent layer consists of rosettes and concentric crystalline circles, with a crystal at the center. There is a space of varying width between the central crystal and

the series of concentric circles. The lines of the patterns are thick and the circles are few in number. The central crystal is larger in the normal pattern. There are also numerous large elongated spear-shaped and needlelike crystals (Fig. 2). The entire field containing all these elements shows an inferior organization to that found in pathologic pictures.

Preparations of pathologic fluids are characterized macroscopically by the fact that empty spaces in the translucent layer do not appear even in dilutions of 1 drop in 10 c.c. or higher dilutions (Fig. 3). Occasionally, with paretic spinal fluid there are no empty spaces even with 1 drop in 20 c.c. At dilutions of 1 drop to 5 c.c., the translucent layer is quite dense and differs also in this

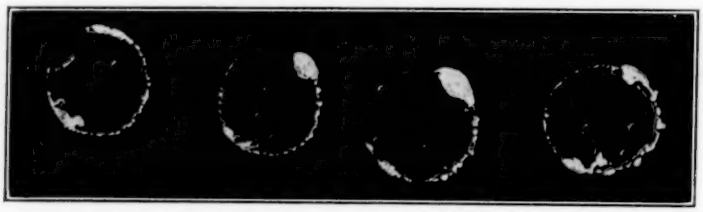


Fig. 1.—Empty spaces surrounded by crystalline circumference in dilutions of 1 drop of normal spinal fluid to 6 c.c. of 0.8 per cent saline.

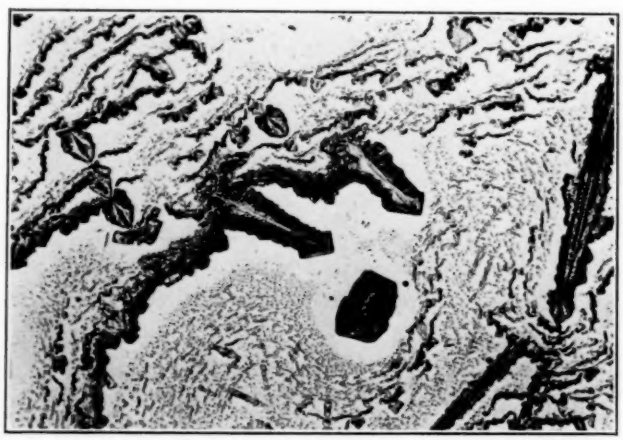


Fig. 2.—Microphotograph of the crystallization of sodium chloride as affected by the addition of 1 drop of normal cerebrospinal fluid to 4 c.c. of saline.

respect from normal pictures at corresponding dilutions. Microscopically the pathologic crystal patterns are characterized by an extremely well-organized design of fine lines, forming concentric circles with a central crystal, parallel lines, parallel needles, or spear-shaped crystals and feather-like crystals, radiating from a central crystal and intersecting concentric lines (Fig. 4).

In certain cases the crystal picture may not be normal and also may not have a definite pathologic pattern described above. Macroscopically, empty spaces may begin to appear only at dilutions of 1 drop to 6 c.c., and there may be a completely empty space only at 1 drop to 10 c.c. Microscopically, there is a mixture of patterns described as normal as well as organized patterns which

are pathologic. Tomesco and associates termed these "mixed" pictures, but it probably were better to consider them as indicating the degree of pathologic change.

DEMENTIA PARALYTICA

Of the 35 specimens of cerebrospinal fluid from patients with paresis studied by this mehod, 33 showed marked pathologic crystal pictures. Macroscopically, the spaces were covered by a translucent crystalline layer in all dilutions; on microscopic examination there were well-organized concentric circles surrounding a central crystal. The central translucence was very dense and in some cases the drop was covered even in dilutions of 1 drop to 20. Two of the specimens

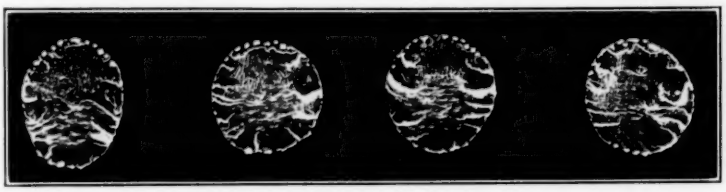


Fig. 3.—Dilution of 1 drop of cerebrospinal fluid from dementia paralytica to 10 c.c. saline. Central translucent layer covered.

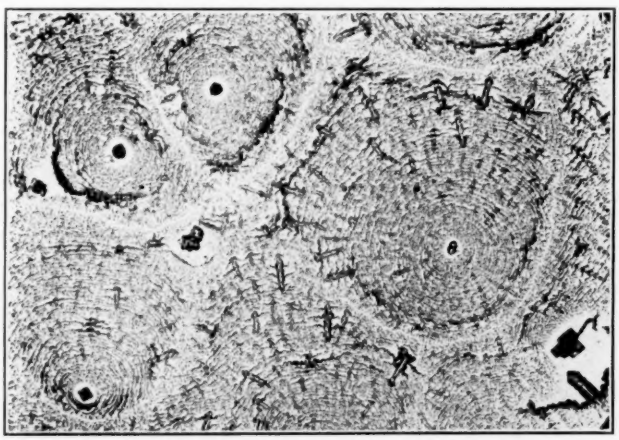


Fig. 4.—Concentric circles surrounding a central crystal showing good organization of the pattern. Crystals from a mixture of 1 drop of cerebrospinal fluid from dementia paralytica and 6 c.c. of saline.

were moderately pathologic as slight open spaces began to appear in dilutions of 1 to 10. These two specimens of spinal fluid showed one-plus globulin by the Pandy reaction. The most extreme pathologic crystal pictures resulted when the contaminating spinal fluid showed a four-plus Pandy or Ross-Jones reaction for globulin. However, the parallelism between the pathologic crystal picture and the intensity of the globulin reaction was not perfect as there were pathologic crystal pictures in specimens in which there was a negative globulin reaction (four preparations).

EPILEPSY

Seventeen specimens of spinal fluid from patients with functional epilepsy were studied. Eleven showed normal patterns. Empty spaces were present at

dilutions of 1 drop to 4 c.c., and microscopic examination indicated a poor organization of the patterns corresponding to the normal picture described above. Six were pathologic to a moderate degree. They showed only slightly open spaces at 1 drop of spinal fluid to 6 and 10 c.c., and there was good organization of the patterns. Of 3 organic cases with epilepsy all showed pathologic patterns. Two were cases of traumatic epilepsy, and one was an institutional epileptic with deterioration.

DEMENTIA PRAECOX

Of the 16 cases of dementia praecox, 10 were normal and 6 were moderately pathologic. All of the spinal fluids from dementia praecox showed negative Ross-Jones and Pandy reactions.

NONNEUROLOGIC DISORDERS

Seventy-one specimens of spinal fluids from diseases other than those of the central nervous system were examined. The diseases included carcinoma of the stomach, hypertension, gastric ulcer, and various surgical cases in which the fluid was obtained before the administration of spinal anesthesia. All of these cases had normal crystal patterns.

DISCUSSION

The factor responsible for the abnormal change in the crystallization of sodium chloride was thought by Tomesco and co-workers to be the albumin and globulin content of the cerebrospinal fluid, because of their ability to lower the surface tension of the solution. This is indicated by a correspondence, though imperfect, of the pathologic changes in sodium chloride crystallization with the classical reactions for albumin and globulin in the cerebrospinal fluid. It was impossible to cause pathologic changes in sodium chloride crystallization by using the filtrate after the colloids were removed from the cerebrospinal fluid by a Seitz ultrafilter. A drop of dilute albumin solution added to 0.8 per cent saline causes changes in sodium chloride crystallization similar to that caused by cerebrospinal fluid. Albumin or a globulin fraction may effect the changes in crystallization, but, on the other hand, pathologic crystal specimens were produced by using bile or saponin, both of which are characterized by the ability to lower the surface tension. It would appear that a substance in the spinal fluid that causes a lowering of the surface tension effects the changes in the crystallization of sodium chloride. Semenchenko and Shikhobalova[10] observed that the crystallization of sodium nitrate is affected but little by salts incapable of changing the surface tension of sodium nitrate. Addition of surface active salts to a solution of sodium nitrate produced considerable effect, causing a greater degree of dispersion of the crystal aggregate than that of pure sodium nitrate. Thus a parallelism exists between the effects on the surface tension of a solution and crystallization of its salts.

SUMMARY AND CONCLUSIONS

The effects on the crystallization of sodium chloride of the contamination of its solution by a minute amount of cerebrospinal fluid were studied. The

crystals formed from solutions to which pathologic cerebrospinal fluid had been added differ from those formed after the evaporation of solutions containing normal cerebrospinal fluid. Pathologic crystal patterns were present, in most to a very marked degree, in all of 35 cases of paresis, in 6 of 17 cases of functional epilepsy, in 3 cases of organic epilepsy, in 6 of 16 cases of dementia praecox, and in various neurospychiatric disorders. Normal patterns were observed in all nonneurologic disorders.

REFERENCES

1. Pfeiffer, E.: Empfindliche Kristallisationsvorgänge als Nachweis von Formungskräften im Blut, Dresden, 1935, Emil Weise.
2. Trumpp, J., and Rascher, S.: Nachprüfung der E. Pfeiffer'schen Angaben über die Möglichkeit einer kristallographischen Diagnostik; Versuch einer Hormonoskopie und Schwangerschaftsdiagnose, München. med. Wchnschr. **83**: 1049, 1936.
3. Mott, F. W., and Halliburton, W. D.: The Chemistry of Nerve Degeneration, Lancet **79**: 1077, 1901.
4. Thompson, W. W. D.: A Note on Certain Peculiar Crystals Found in the Cerebrospinal Fluid From a Case of Septic Meningitis, Lancet **188**: 653, 1915.
5. Levinson, A.: The Cerebrospinal Fluid in Health and Disease, St. Louis, 1919, C. V. Mosby Co.
6. Henning, N., and Beck, A.: Liquordiagnostik am Eintrocknungsbild, Klin. Wchnschr. **13**: 251, 1934.
7. Keller, C. J., and Schoen, H.: Fluorescenzuntersuchungen am Eintrocknungsbild des menschlichen Liquor cerebrospinalis, Klin. Wchnschr. **14**: 817, 1935.
8. Zeiner-Henriksen, K.: Crystal Formations in the Spinal Fluid and Their Diagnostic Significance, J. Neurol. & Psychopath. **16**: 111, 1935.
9. Tomesco, P., Cosmulesco, I., and Serban, F.: Une propriété inconnue du liquide céphalo-rachidien: la chloruro-cristallisation. Les aspects normaux, Bull. Soc. de psychiat. de Bucarest **1**: 11, 1936; La chloruro-cristallisation dans la paralysie generale. Les aspects pathologiques de cette propriété du liquide céphalo-rachidien, Bull. Soc. de psychiat. de Bucarest **1**: 38, 1936; La chloruro-cristallisation dans les maladies mentales non syphilitiques, Bull. Soc. de psychiat de Bucarest **1**: 52, 1936; Formations cristallines du liquide céphalo-rachidien et chloruro-cristallisation, Bull. Soc. de psychiat. de Bucarest **2**: 122, 1937.
10. Semenchenko, V. K., and Shikhobalova, L. P.: The Effect of Impurities on Crystallization, Mineral Suir'e **11**: 27, 1936. (Chem. Abst. **30**: 7014, 1936.)

SENSITIZATION IN THE CONVULSIVE STATES WITH SPECIAL REFERENCE TO HETEROPHILE ANTIGEN*

I. Heterophile Hemolysins

Edward W. Lazell, M.D., Northport, N. Y.

OBJECT OF THE RESEARCH

THE purpose of this article is to report the investigation of heterophile hemolysins in a group of 36 veterans suffering with idiopathic epilepsy. It is the first of a series of papers devoted to the various phases of sensitization with heterophile antigen occurring in persons having convulsions.

This study of heterophile sensitization was made to investigate the hypothesis that "sensitization with heterophile antigen is the basis of many clinical forms of nervous and mental disease," a concept which developed slowly and which is dependent upon factors so little known to the profession that considerable introductory discussion of heterophile sensitization is necessary for a better understanding of our results.

Heterophile sensitization may prove of great importance not only in nervous and mental disease but also for immunity in general. Because of the many ramifications found and the difficulties encountered, stating definite conclusions would often have been hazardous. We shall, therefore, present the factual study and the methods used with the hope that the reports will interest other workers in this subject and then emphasize that even the opinions expressed and the conclusions drawn are wholly tentative and may be revised by future observations and experience. It will remain for other workers to prove or disprove them.

The development of the concept of "heterophile sensitization" is of itself of considerable interest so that the coordination of all of those conclusions which indicate the importance of the heterophile factor, not only in nervous and mental disease but also for immunity in general, are of far greater importance than the study of any single factual manifestation of heterophile sensitization. These broader implications which indicate the definite value of heterophile sensitization in nervous and mental disease and for immunity in general the reader will be forced to gather from the series of articles or by reference to later articles in which they will be summarized.

*From the Neuro-Psychiatric Research Unit for the Study of the Influence of Heterophile Antigen in Nervous and Mental Disease, Veterans' Administration Facility, Northport. Article No. 3.

Published with the permission of the Medical Director who assumes no responsibility for the opinions expressed or conclusions drawn.

Received for publication, January 17, 1938.

The philosophical point of view under which these cases were studied was that of the body/mind as previously outlined by the writer.[1, 2] This special concept of the body/mind is indeed the coordinated unity stressed by psychiatry during the past twenty years. This concept predicates that whatever changes the body, thereby alters the mind; and whatever modifies the mind also influences the body because the mind is the collective molecular vibration of the body or soma, and is inseparable from it. This concept is best illustrated by an atom of hydrogen: substance in vibration. The substance is inseparable from the vibration, and the vibration from the substance. This concept of matter when applied to the human problem sees the body and mind as the two poles of the same thing. The collective molecular activities of the soma (substance) are arranged in functions, one of which *is* the mind.

The scientific data which supported this hypothesis and led to the formation of the research were presented to the American Psychiatric Association in a paper read on May 13, 1935.[3] These studies began in 1928 with the investigation of the influence of the blood of dementia praecox on frogs' polliwogs, the first report of which appeared in February, 1929.[4] This preliminary communication reported that whereas the blood serum of normal males in a dilution of 1:1,000 sustained the life and growth of young frogs' polliwogs, the blood serum of patients suffering from dementia praecox in dilution of 1:1,000 killed the young polliwogs in three days or less, and every polliwog exposed to such serum died within a short time.

While trying to explain the phenomena shown in the polliwog experiment, our attention was casually directed to the existence of heterophile antigen. In this manner the toxic or lethal factor in the blood of epilepties and heterophile antigen became associated in our minds. A résumé of the outstanding features of heterophile antigen, as collected from the literature, was made at that time.[5] The salient features of this abstract follow:

Prior to 1911, immunologists believed in the general law of species specificity. That is to say, the tissue of an animal or of a parasite when injected into another animal produced an antibody which reacted only with the substance by which it was produced: The antibody was homologous. In 1911, however, Forssman impressed upon the profession the fact that the assumption of the general law of species specificity was not justified. His work had been anticipated by other workers, such as Frouin, who immunized rabbits with an acetone extract of the yolk of chicken eggs and obtained a lysin for the red corpuscles of a dog. Forssman injected rabbits with kidney, suprarenal gland, liver, testicle and brain of guinea pigs and obtained a lysin for the red blood cells of a sheep. The tissue of the guinea pig, when injected into the rabbit, produced an antibody which was heterologous; that is to say, the antibody so generated had an affinity for the receptors of a species other than those in response to which it developed. This heterologous antibody in time became known as "heterophile."

Following Forssman, many investigators studied heterophile antigen, with the result that it was found to be widely distributed in the animal and plant

kingdom. Animals were divided into two large groups: (a) those which produce heterophile antigen and (b) those which do not. Organs of the guinea pig (except the blood corpuscles), horse, dog, cat, mouse, fowl, tortoise, several kinds of fish, horse urine, and some bacteria when injected into rabbits contain heterophile antigen which produces antisheep hemolysin in the blood of the rabbit. These animals are said to be of the guinea pig type. Organs of the ox, sheep, rabbit, pig, man, rat, goose, pigeon, frog, and eel when injected into rabbits do not contain heterophile antigen for the production of antisheep hemolysin. They are said to belong to the rabbit type. As time went on, the whale, camel, and ostrich, together with sheep's sperm, mouse cancer, gills of the carp and pike, urine of the horse and guinea pig, lens of the guinea pig and the horse, and the paratyphoid (some) and Gaertner bacillus were found to contain heterophile antigen. Attention is directed to the fact that *man is in the rabbit group, whereas the horse, chicken, and fowl and the egg of the chicken are in the guinea pig group.*

In general it was found that those animals whose tissues contain heterophile antigen will not produce heterophile antibodies, and that those animals which contain heterophile antigen in their tissues do not contain it in their red corpuscles. The chicken, however, is an exception to this rule, *both the tissues and the blood of the chicken producing heterophile antibodies* when injected into the rabbit.

Taniguchi[6] investigated the subject of heterophile antigen very thoroughly and found that certain tissues from a variety of animals when injected into the rabbit had the property of generating hemolytic immune bodies for sheep's corpuscles; that this heterogenic antigen was not equally distributed in different organs of the same species, but was very strong in guinea pig kidney; that the receptors of heterogenic antigens reside in the lipoids of the tissues, especially those soluble in alcohol and ether but insoluble in acetone—that is, the so-called "lecithin" fraction.

He demonstrated that heterophile antibodies have four characteristics: (1) they combine with lipoids derived from heterogenic antigen; (2) they fix the complement in the presence of such lipoids; (3) they form precipitates when combined with the emulsions of the lipoids, and (4) they are heat stabile: boiling does not destroy them. The hemolytic power of such an antiserum is always parallel with its precipitating power and its complement fixing properties in a quantitative sense. The specific heterogenic reactions are intensified by the addition of cholesterol and also by increasing the turbidity of the antigen emulsion.

Fortunately, our knowledge of heterophile antigen is increasing rapidly. One of the most important observations made was that heterophile antibodies can be produced by the alimentary route; that is, by feeding the animal with heterophile tissues. G. Howard Bailey,[7] from the Department of Immunology of Johns Hopkins, reported that heterophile antibodies in rabbits could be produced by the intranasal feeding with *B. lepisepticum.*

I. Davidsohn[8] studied the four blood types to determine the presence of heterophile antigen. Types O, B, and AB did not show the presence of heterophile antigen, while rabbits injected with the blood of Type A developed heterophile immune serum; but *these antibodies were not identical with those produced by injecting sheep corpuscles!* Davidsohn,[9] by means of absorption experiments, established the heterophile nature of the agglutinins and hemolysins found in the serum of patients with horse serum sickness; Harry H. Donnally[10] showed that egg white appeared in human milk; Francis E. Holford[11] found that egg albumen, horse or beef serum, the pseudoglobulin fraction of beef serum, and the globulin fraction of horse serum, injected into pregnant rabbits near term, passed the placental barrier in sufficient quantities to be demonstrable in the serum of the fetus; E. M. Butt and A. G. Foord[12] found a high titer of heterophile antibodies in infectious mononucleosis. John R. Paul and W. W. Bunnell[13] found a very high titer of heterophile antibodies in infectious mononucleosis.

The importance of heterophile antigen when considered in the light of the above researches is startling. It means that under certain conditions a pregnant woman may sensitize her unborn child by eating chicken and eggs; that children may be sensitized by mother's milk and cow's milk; that children may be sensitized by food or air-borne substances, such as dog and cat dander, and later show allergic reactions in a multitude of ways. More particularly, it means that a person may be sensitized to heterophile antigen (perhaps from eggs or chicken) and later react to *any substance which contains heterophile antigen.*

But we must look upon the heterophile problem as a group reaction rather than as a single definite entity.

About this time our attention was drawn to the work of Jonathan Forman[14] who found epileptiform attacks following sensitization by a large number of substances, many of them heterophile, and relieved the patients by desensitization methods; L. P. Howell[15] reviewed the work which has been done on desensitization in epilepsy and added his own cases successfully treated. Many others have contributed to our knowledge of allergy, a review of which by Francis M. Rackemann[16] recently appeared; also *Clinical Allergy* by Rowe.[17]

Meanwhile the problem of hypersensitiveness or anaphylactic shock was being studied with the well-known results that the first injection sensitized the animal so that a second dose administered after an interval of time killed it. But the mechanism producing death varied in the different species: The guinea pig died of asphyxia due to spasm of the bronchioles; the rabbit, of cardiac failure with obstruction of the pulmonary circulation and dilatation of the right side of the heart; the dog, from capillary dilatation, fall of blood pressure and enormous distention and congestion of the liver and abdominal viscera; and rats could not be made anaphylactic at all. Kolmer[18] describes the symptoms of immediate and severe anaphylactic shock as follows: "These are characterized by sudden onset—often within a few minutes after or during the intravenous

injection of serum. The patient becomes restless, anxious, and may give an outery; there is marked pallor, perspiration, rapid and feeble pulse. Respirations become deep, labored and sometimes rapid, and the patient may become unconscious. Muscular twitchings, rigors, and convulsions may occur with micturition and defecation. In rare instances death occurs during coma with respiratory failure, while the heart is still beating.'' (The relation between the epileptiform convulsion, anaphylactic shock, and insulin hypoglycemia will be discussed in a later article.)

Looking at the problem of hypersensitiveness in man as an allergy, it presented a quite different set of problems from anaphylaxis in animals, most of which are still unsolved. Many persons were found to be hypersensitive when no previous parenteral source of antigenic supply could be found. Heredity is apparently an important factor. Its influence in the production of a sensitiveness has been explained as an inability to produce an immunity. (See explanation of Reuben S. Kahn.[19])

The study of hypersensitiveness in man has forced a broadening of the concept of what constitutes anaphylaxis. Antigens have at least five distinct qualities: the formation of (a) hemolysins; (b) precipitins; (c) agglutinins; (d) skin sensitizing bodies and, finally, the *sensitization of unstriped muscle*. Consequently, we must regard as anaphylactic phenomena not only the commonly accepted fatal ''shock'' occasionally seen, but also many other phenomena in which one of the five qualities of antigens enters as the mechanism. We must regard as anaphylactic the nasorespiratory sensitizations in asthma and hay fever; the cavity formation in tuberculosis; the hemolytic secondary anemias due to hemolysins; the skin reactions seen in the food eruptions especially of children, some forms of eczema, urticaria, angioneurotic edema, erythema multiforme, and after eating fruits such as strawberries; many forms of disease due to agglutinins, such as thrombosis; and finally many conditions due to sensitization of unstriped muscle and the unstriped muscle component of the skeletal muscle, such as temporary ischemias and catatonic rigidity. (Acute cardiac collapse and heart failure in the absence of myocarditis and coronary disease fall in this class and will be discussed in a later article.)

It is at once apparent that the concept of heterophile sensitization is a fusion of two theories: (a) the specific influence of heterophile antigen, and (b) sensitization (allergy) or anaphylaxis.

It was with this understanding of allergy due to sensitization with heterophile antigen that a small group of epilepties was studied in the spring of 1935.

The material consisted of 14 veterans, all committed as insane to the Veterans' Administration Facility at Northport, L. I., N. Y. There was no selection in these cases. They had developed convulsions at all ages from infancy to adult life, gave the usual history of childhood conflicts and inadequacies, head traumas and infections, and showed varying degrees of deterioration.

The first observation was a clinical study of the occurrence of convulsions in relation to certain articles of food. It was found that certain patients had convulsions in definite relation to the ingestion of foods such as chicken, flounder, gelatin, pork, eggs, etc.

At this point an attempt was made to extend the list of the then known heterophile antigen containing foods by the intraperitoneal injection of rabbits with saline emulsions of salmon, haddock, flounder, cod, gelatin and pork chop. The serum of these rabbits, which before injection had given negative results, now gave positive complement fixation tests with saline emulsion of guinea pig kidney and showed a high titer of hemolysins for sheep's red cells. This explained the results recorded on the food diaries and indicated that the foods causing the attacks contained heterophile antigen.

As a control for our cases, specimens of blood of 29 patients suffering from convulsions kindly supplied by the authorities at the Kings Park State Hospital were studied. The sera came to our laboratory in numbered vials, the names of the patients being withheld. There were a number of discrepancies in our results; but a later study of the clinical folders explained these discrepancies by showing that these were cases of convulsions occurring in syphilis, encephalitis, or intracranial hemorrhage at birth. When these latter cases were excluded, the results in the control group were very similar to those in our series.

The connection between the convulsions, as recorded in the food diary, and the sensitization of the individual patient to the corresponding food, was now shown by scratch and intracutaneous tests made on the group. The results proved that the epileptic is heavily sensitized to certain foods; and the cause and effect relationship between the ingestion of a food to which the patient was sensitive and the occurrence of the convulsion was definitely established.

In our report of this preliminary work to the American Psychiatric Association in the paper read on May 13, 1935, the thesis was advanced that:

"(a) The cases of iodiopathic epilepsy and of dementia praecox which we examined were persons sensitized to heterophile antigen.

(b) These diseases represent an atopy (allergy), with hereditary or infantile factors.

(c) The convulsive attacks, mental equivalent and *disturbed visceral states* (see especially our later study of hypoglycemia) followed the ingestion of excessive amounts of heterophile antigen-containing foods."

The results of this preliminary study are noted on Table I. It was to investigate the results of this study that the research at Northport was initiated.

MATERIAL AND METHODS

In order to have sufficient clinical material for the research, voluntary patients were transferred from the Veterans' Administration Facility at Lyons, N. J. The group finally totaled 36. There was no selection of these cases,

TABLE I

NUMBER	HEMOLYSINS	AGGLUTININS	ADSORPTION OF HEMOLYSINS (PER CENT)	COMPLEMENT FIXATION USING ALCOHOL ANTIGEN	COMPLEMENT FIXATION USING SALINE EMULSION AS ANTIGEN	BLOOD TYPE
1	1:4 C 1:8 50 per cent		100 50 0 0	1. * 2. ** 3. ***	1. Negative 2. ** 3. **	O
2	1:8 C 1:16 75 per cent		50 25 0 0	1. * 2. ** 3. **	1. Negative 2. *** 3. ****	A
3	1:4 C 1:8 75 per cent		100 100 50 0	1. ** 2. ** 3. **	1. Negative 2. ** 3. ***	A
4	1:8 C 1:16 50 per cent		100 100 50 25	1. *** 2. *** 3. ***	1. **** 2. **** 3. ****	O
5	0		0 0 0 0	1. ** 2. *** 3. ***	1. ** 2. ** 3. ***	A
6	1:8 C 1:16 50 per cent	1:4 C	100 50 0 0	1. * 2. ** 3. **	1. ** 2. *** 3. ****	A
7	1:4 C 1:8 50 per cent		50 0 0 0	1. * 2. ** 3. **	1. **** 2. **** 3. ****	O
8	1:16 C 1:32 25 per cent		100 100 50 0	1. ** 2. *** 3. ***	1. **** 2. **** 3. ****	A
9	1:4 C 1:8 75 per cent	1:4 C	50 0 0 0	Not tested	1. **** 2. **** 3. ****	A
10	1:8 C 1:16 50 per cent	1:4 C	100 25 0 0	1. * 2. ** 3. **	1. Negative 2. * 3. ***	A
11	0		0 0 0 0	1. * 2. ** 3. **	1. Negative 2. ** 3. **	O
12	1:4 C 1:8 75 per cent	1:4 C	50 0 0 0	1. Negative 2. ** 3. **	1. Negative 2. *** 3. ****	AB
13	1:8 C 1:16 50 per cent	1:4 C	100 100 0 0	1. * 2. ** 3. **	1. *** 2. **** 3. ****	B
14	0		0 0 0 0	1. Negative 2. * 3. *	1. Negative 2. Negative 3. Negative	A

C = Complete.

except that those showing convulsions due to syphilis or alcoholism were excluded. In the group there were four frank cases of dementia praecox with convulsions.

PREPARATION OF MATERIAL USED IN TESTS

Alcoholic Kidney Antigen (guinea pig and ox).—The kidneys were removed from healthy, male guinea pigs, capsules and fat removed, and the kidneys weighed. Then minced and ground in a glass mortar. One part of minced tissue (free from fat) was added to 9 parts of 96 per cent alcohol. The mixture was allowed to stand at room temperature for from seven to ten days, being shaken at frequent intervals. The clear extract was then filtered through fat-free filter paper and kept in the dark in stoppered bottles.

Ox kidney antigen was prepared by the same method as above.

Saline Cell Suspension Antigen (guinea pig and ox).—A portion of kidney with the capsule removed was weighed, minced, and ground in a glass mortar. About 10 volumes of saline was added, and the mixture filtered through gauze. The suspension was washed with saline (4 to 6 times) until the supernatant fluid became perfectly clear. One volume of the sediment was then resuspended in four volumes of normal saline. This antigen was always used the same day it was prepared.

Heterophile Antibody.—For generating heterophile antibody 1 part of well minced guinea pig kidney was added to 9 parts of normal saline solution. After thoroughly macerating in a mortar and pestle, the mixture was filtered through gauze to remove the coarse particles. One cubic centimeter of the filtrate was injected intraperitoneally into rabbits. Immune body for sheep's corpuscles: the standard method of generating immune body for the corresponding sheep's corpuscles was used.

Suspension of red blood corpuscles—0.5 c.c. of a 3 per cent suspension of washed sheep blood corpuscles was used.

Complement—0.05 c.c. of guinea pig's complement was used.

The hemolytic system was titrated as for a standard Wassermann.

Antisheep and Anti-ox Amboceptor.—Healthy male rabbits, weighing from 4 to 5 pounds, were injected at five-day intervals with a washed 10 per cent suspension of corpuscles. One-half cubic centimeter of the cell suspension was given at the first injection. This dose was increased at each subsequent injection by 0.5 c.c. until at the fifth and last dose the rabbit was given 2.5 c.c. These injections were given intravenously, the marginal vein of the ear being used. Five days after the fifth dose, about 5.0 c.c. blood was collected from the ear vein and a preliminary titration done. If found to be of sufficient titer, the rabbits were then anesthetized and bled from the heart.

The blood was then set in the refrigerator until clot formed, centrifuged, the serum removed, and heated in the water bath at 56° C. for half hour. This serum then diluted 50 per cent with sterile glycerin. The tubes stoppered with paraffin stoppers and preserved in the icebox.

Sterile precautions throughout the preparation of these amboceptors were observed. Check titrations were done at frequent intervals to be sure of the titers on these amboceptors.

PROCEDURE

Technique of Sheep Cell Hemolysins—used by John R. Paul and co-workers to determine the presence of heterophile antibodies in infectious mononucleosis:

1. Original dilutions of 0.5 c.c. of inactivated sera (fifteen minutes at 55° C.). Dilutions from 1:4 or higher set up in 0.5 c.c. portions.

2. To these, 1 c.c. of guinea pig complement in a dilution of 1:30 added.

3. 0.5 c.c. of a 2 per cent suspension of sheep cells added.

4. 1 c.c. of saline added to bring total to 3 c.c.

5. Shake, place one hour in water bath at 37.5° C. and read.

Lytic Test.—1. Patient's serum inactivated at 56° C. for thirty minutes.

2. Dilutions prepared from 1:2 to 1:80, inclusive, of above inactivated blood.

3. To a series of test tubes, 0.5 c.c. of the respective dilutions were added.

4. Then to each tube was added 0.5 c.c. of a 2 per cent suspension of corpuscles (sheep or ox).

5. Then to each tube was added 1 c.c. of a 1:30 dilution of treated guinea pig complement.

6. 1 c.c. normal saline was added to each tube.

7. Incubated for one hour at 37.5° C.

8. Preliminary reading made. The racks set in the icebox to remain overnight.

9. Read and recorded in percentage of hemolysis.

Controls for Above Test.

1. Cells only.

2. Complement + cells.

3. Amboceptor + cells.

4. Complement + amboceptor + cells.

*Adsorption Test.**—Using saline cell suspensions of guinea pig and ox kidney.

1. Patient's serum inactivated at 56° C. for thirty minutes.

2. 0.6 c.c. of above inactivated serum added to 0.6 c.c. of 1:4 suspension of washed guinea pig or ox kidney cells, in saline.

3. Incubated at 37.5° C. for one hour, shaking every fifteen minutes.

4. Tubes then centrifuged, the supernatant fluid removed, which is now a dilution of 1:2.

5. 0.5 c.c. of this dilution added to a serologic tube.

6. 0.5 c.c. of a 2 per cent suspension of sheep or ox corpuscles added.

7. 1.0 c.c. of 1:30 dilution of treated guinea pig complement added.

8. Tubes shaken and then incubated for one hour at 37.5° C.

9. Preliminary reading made and racks set in icebox for an overnight reading.

10. Tubes read and reported the following morning in percentages of hemolysis inhibition.

Controls for Above Test.

1. Cells only.

2. Patient's serum + cells.

3. Kidney cell suspension + cells.

4. Complement + cells.

5. Complement + kidney cells + cells.

6. Complement + patient's serum + cells.

7. Complement + amboceptor + cells.

Adsorption Test.†—Using saline emulsions of alcoholic extract of guinea pig and ox kidney.

1. Patient's serum inactivated at 56° C. for thirty minutes.

2. Add 0.5 c.c. of 1:2 dilution of the above serum to a serologic test tube.

3. Add 0.5 c.c. of 1:10 dilution in saline of alcoholic organ extract. (Add saline to the measured quantity of antigen slowly, shaking while mixing to obtain the maximum amount of turbidity.)

4. Incubate at 37.5° C. for one and one-half hours.

5. Add 0.5 c.c. of a 2 per cent suspension of sheep or ox corpuscles.

---

*Inhibited lytic test using saline suspension of kidney cells.
†Inhibited lytic test using alcoholic antigen.

6. Digest for one hour in icebox.

7. Centrifuge and discard the supernatant fluid.

8. Resuspend corpuscles in 1.0 c.c. of saline.

9. Add 1.0 c.c. of 1:30 dilution of treated guinea pig complement.

10. Incubate for one hour at 37.5° C.

11. Make preliminary reading, set in icebox for overnight reading.

12. Read and record the following morning.

Controls for Above Test.

1. Cells only.

2. Patient's serum + cells.

3. Antigen + cells.

4. Complement + cells.

5. Complement + antigen + cells.

6. Complement + patient's serum + cells.

7. Complement + amboceptor + cells.

Agglutination Test.—1. Patient's serum inactivated at 56° C. for thirty minutes.

2. Dilutions prepared from 1:2 to 1:80, inclusive, of above inactivated blood.

3. To a series of test tubes, 0.5 c.c. of the respective dilutions were added.

4. Then to each tube was added 0.5 c.c. of a 2 per cent suspension of corpuscles (sheep or ox).*

5. Then to each tube was added 1.0 c.c. of normal saline.

6. Incubated at 37.5° C. for one hour.

7. Set in the icebox, to be read the following morning.

8. The mouth of the tube closed with the finger and tube gently turned upside down three times; then observed for agglutination.

Agglutination that is noted macroscopically was recorded as positive. The cells were set up in saline as a control. Would suggest microscopic examination of all tubes showing no macroscopic agglutination.

Complement Fixation.—Using alcoholic guinea pig and ox kidney antigen.

1. 0.25 c.c. of patient's serum inactivated at 56° C. for thirty minutes added to a series of five tubes, respectively.

2. To the first four tubes was added 0.5 c.c. of a 1:10 suspension of alcoholic kidney antigen in saline. No antigen was added to tube 5 which is the control tube.

3. To tubes 1, 2, 3, and 4 was added, respectively, 1, 2, 3, and 4 M.H.D.† of guinea pig complement (treated). To tube 5, 3 M.H.D. of complement was added.

4. The tubes then shaken and incubated for one hour at 37.5° C.

5. To each tube, 0.5 c.c. of anti-ox amboceptor was added which had been previously titrated and adjusted to where this dose contained two units.

6. To each tube was added 0.5 c.c. of a 2 per cent suspension of ox corpuscles.

7. The tubes then shaken and incubated for one hour at 37.5° C.

8. Preliminary reading made; tubes set in icebox for overnight reading.

9. Test read and recorded the following morning.

Controls for Above Test.

1. Cells only.

2. Amboceptor + cells.

3. Complement + cells.

4. Antigen + cells.

5. Antigen + complement + cells.

6. Antigen + complement + amboceptor + cells.

*Cells were allowed to age for seventy-two hours before using in an agglutination determination. It has been reported by different men that the maximum amount of agglutination will not be obtained with very fresh corpuscles.

†Minimum hemolytic dose.

TABLE IIA

CASE NO.	REMARKS	HEMOLYSINS												AGGLUTININS				BLOOD TYPE
		STRAIGHT LYTIC				TREATED WITH CELL SUSPENSION				TREATED WITH ALCOHOLIC ANTIGEN				STRAIGHT AGGLUTININ				
		SHEEP		OX		GUINEA PIG KIDNEY CELLS		OX KIDNEY CELLS		GUINEA PIG KIDNEY		OX KIDNEY		SHEEP		OX		
		Upper	*Lower*	*Upper*	*Lower*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Upper*	*Lower*	*Upper*	*Lower*	
1	Grand mal—No convulsion for 1½ yr. (Note—Isophile hemolysins, column 5)	C 1-16	Tr 1-128	Tr 1-12	Sl. Tr 1-24	AC 1-2	O 1-2	C 1-2	O 1-2	C 1-2	C 1-2	Tr 1-2	AC 1-2	O 1-2	O 1-2	O 1-2	O 1-2	O
2	Grand mal at infrequent intervals	C 1-4 C 1-2 20% 1-2	28% 1-48	Tr 1-12		O 1-2	O 1-2	AC 1-2	O 1-2	AC 1-2	50% 1-2	C 1-2	65% 1-2	O 1-2	O 1-2	O 1-2	O 1-2	A
3	Grand mal at frequent intervals	C 1-6 C 1-2 C 1-2 C 1-2		50% 1-2 Tr 1-2 O 1-2 M 70%	60% 1-6	O 1-2	O 1-2	C 1-2 C 1-2	O 1-2	C 1-2 C 1-2	C 1-2 C 1-2	C 1-2 C 1-2	C 1-2 C 1-2	++++ 1-4	+1-6	++ 1-2	+1-4	A
4	Grand mal at infrequent intervals	C 1-8 C 1-2	Tr 1-56	O 1-2 AC 1-2	O 1-2	O 1-2	O 1-2	AC 1-2 C 1-2	O 1-2	85% 1-2	85% 1-2	75% 1-2	85% 1-2	O 1-2	O 1-2	+1-4	+1-8	O
5	Grand mal formerly, now petit mal	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2	O 1-2	O 1-2	O 1-2	O 1-2 25% 15% 1-2	M 25% 1-2 15% 1-2	O 1-2 10% 1-2	M 1-2 10% 1-2	O 1-2	O 1-2	O 1-2	O 1-2	A
	Two attacks in 1936, recovering	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2					O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2					

C = Complete.
AC = Almost complete.
Tr = Trace.
M = Marked.

TABLE IIA—CONT'D

6	Petit mal since childhood at infrequent intervals Emphysematous	C 1-6 M 50% C 1-2		O 1-2 O 1-2 Tr 25% 1-2	O 1-2	O 1-2	O 1-2	AC 98% 1-2 C 1-2	O 1-2	80% 1-2 C 1-2	99% 1-2 C 1-2	C 1-2 C 1-2	C 1-2 C 1-2	++++ 1-2	+1-6	O 1-2	O 1-2	A
7	Petit mal at infrequent intervals	C 1-2 O 1-2 C 1-2 C 1-2	Sl. Tr 1-8	O 1-2 50% 1-2 O 1-2 Tr 1-2	O 1-2	O 1-2	O 1-2	C 1-2	O 1-2	85% 1-2	75% 1-2	85% 1-2	90% 1-2	O 1-2 +++ 1-2	O 1-2	O 1-2	O 1-2	O
8	Petit mal at infrequent intervals	C 1-20 C 1-2 C 1-2 C 1-2	20% 1-96	Tr 1-20 Tr 1-2 O 1-2 Tr 1-2	O 1-28	O 1-2	O 1-2	C 1-2	O 1-2	C 1-2 AC 98% 1-2	AC 95% 1-2	C 1-2 C 1-2	C 1-2 C 1-2	O 1-2	O 1-2	O 1-2	O 1-2	A
9	Petit mal at infrequent intervals (Note—Isophile hemolysins, column 5)	C 1-16 M 75% 1-2 C 1-2 C 1-2 C 1-2	90% 1-20	C 1-2 M 80% 1-2 75% 1-2 AC 1-2 C 1-2	94% 1-20	M 25% 1-2 M 1-2	O 1-2 O 1-2	AC 95% 1-2 AC 1-2	O 1-2 O 1-2	AC 95% 1-2 M 50% 1-2	C 1-2 C 1-2	C 1-2 C 1-2	C 1-2 C 1-2	++++ 1-2 +1-4	O 1-6	+1-2	O 1-6	A
10	Petit mal at infrequent intervals	C 1-16	AC 98% 1-20	O 1-2	O 1-2	O 1-2	O 1-2	C 1-2	O 1-2	98% 1-2	40% 1-2	98% 1-2	40% 1-2	O 1-2	O 1-2	O 1-2	O 1-2	A
11	Petit mal almost daily	Sl. Tr 1-6 O 1-2 O 1-2		O 1-2 O 1-2 O 1-2	O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2	O 1-2	O 1-2 O 1-2	30% 1-2	M 1-2 O 1-2	60% 1-2	O 1-2	O 1-2	O 1-2	O 1-2	O
12	Petit mal at infrequent intervals, hay fever	Sl. Tr 1-4 Sl. Tr 1-2 O 1-2 O 1-2 Sl. Tr 1-2	Sl. Tr 1-6	O 1-2 O 1-2 O 1-2 O 1-2 O 1-2	O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	M 1-2 20% 1-2	O 1-2 O 1-2	O 1-2 O 1-2 O 1-2 O 1-2 Tr 1-2	M 1-2 Tr 1-2 Tr 1-2 O 1-2 M 80%	C 1-2 90% 1-2 AC 1-2 M 1-2 C 1-2	M 1-2 Tr 1-2 Tr 1-2 O 1-2 M 50%	O 1-2	O 1-2	O 1-2	O 1-2	AB

FACTUAL INVESTIGATION OF THE PRELIMINARY GROUP

In the spring of 1935 a rabbit was injected intraperitoneally with an emulsion of guinea pig kidney. After a week, the serum of this rabbit was examined and found to contain substances lytic for sheep corpuscles. This serum was then tested with alcoholic extract of guinea pig kidney and found to fix the complement, thus identifying the antigen as heterophile.

The serum of patients was now substituted for the rabbit's serum. The result of the lytic test on the patient's serum is shown in column 1, Table I. Other workers have found that the hemolysins parallel the agglutinins so closely that tests for the former have been abandoned. In the examination of 450 cases with no history of serum sickness, Davidsohn (loc. cit.) found that only 4.2 per cent had agglutinins, and these in the lowest dilutions 1:4. Eleven of our cases, therefore, showed sheep hemolysin in excess of normal or 78 per cent; three were negative. These bloods were repeatedly tested but these three remained negative, a fact which is discussed later.

Five of these serums were examined for agglutinins. All were positive as shown in column 2, Table I.

Absorption tests were then made on these serums after first fixing the hemolysin by heating it with alcoholic extract of fresh guinea pig kidney. The positive test is shown by failure to lyze the sheep corpuscles. The blood of two physicians used as negative controls showed 100 per cent failure to prevent lysin of sheep corpuscles, whereas the blood of the immunized rabbit gave 100 per cent inhibition in all four dilutions. Here again the serums which failed to show hemolysis in the straight lytic test were inactive—only three of the four being tested. The results of this test are seen in column 3, Table I.

The next step was the complement fixation test. A rabbit was inoculated intraperitoneally with saline emulsion of guinea pig kidney. After an interval, the serum of this rabbit was run in complement fixation tests, using alcoholic extract of guinea pig kidney as antigen. The serum was run in dilutions 1:4, 1:8, etc. up to 1:1,024. There was slight hemolysis in the first two tubes, but none in the remainder. The complement was bound. The control contained no rabbit serum and there was no hemolysis.

The serum of patients was now substituted for the rabbit's serum with the results appearing in column 4, Table I. The antigen was an alcoholic extract of guinea pig kidney. The antigen control resulted in no hemolysis while the positive control, the serum of a rabbit immune to guinea pig kidney, gave all three tubes four-plus positive. In reading the results one plus was 25 per cent, and so on. One-half cubic centimeter of graduated amounts of complement 1:10, 1:15, and 1:20 were used.

On checking these results the controls failed and it was found that the alcoholic antigen caused an anticomplementary reaction. An alcoholic extract of dry antigen also failed and we turned to the saline emulsion of guinea pig kidney made fresh on the day of the test. We also changed to graduated amounts of serum instead of guinea pig complement. Our results agreed with those reported by E. M. Butt and A. G. Foord (loc. cit.). They did not find

that a high concentration of heterophile antibodies affected the complement fixation. The results of the tests, using saline emulsion of guinea pig kidney as antigen, are shown in column 5. The tubes contained 0.05 c.c., 0.1 and 0.15 c.c. of a 1:100 dilution of human serum. The control tube contained no antigen and gave no hemolysis. The serum of normal human controls gave negative results; that of a patient who had recently been given antitetanic horse serum was heavily positive; that of one with an anxiety neurosis, negative.

Lytic tests were next made on the boiled urine. The interpretation of these results will require a special study; but it is interesting to note that complement fixation was positive in two cases indicating that *heterophile antibody is excreted via the urine*. This fact may possibly be made use of in diagnosis.

FACTUAL INVESTIGATION OF THE RESEARCH GROUP

When the research to investigate these results was started in October, 1935, the work of T. Taniguchi,[20] together with that of other workers on heterophile antigen, was repeated. Rabbits were injected intraperitoneally with saline emulsions of common animal, fowl, fish, and vegetable tissues to determine if the rabbits so injected would develop heterophile antibodies. In this manner, the results obtained by Taniguchi were confirmed and our methods perfected.

We then proceeded with the study of the blood of patients; but when human blood was substituted for the blood of rabbits, we found, as other workers had previously done, important differences between animal and human serum. The following conclusions seemed justified and are cited at this time because they modified the subsequent work:

The serologic conditions produced in animal experiments did not parallel conditions found in human serum, and conclusions reached after animal experimentation could not be applied to human serum in the study of heterophile antibodies. The blood of human beings does not normally contain the same natural hemolysins and other immune bodies as do the animals, so that various modifications of the technique were found necessary when applying the tests to human blood. Taniguchi's work with rabbits was not confronted with the problem of a high titer of natural sheep hemolysins. Our work confirmed the statement by Kolmer[21] to the effect that "in over 80 per cent of human serums there is present sufficient natural amboceptor for sheep cells to give well marked or complete hemolysis." We were, therefore, confronted by the problem of showing not that the blood of epileptic patients contained hemolysins for the sheep's corpuscles but that these hemolysins were heterophile in nature and not isophile.

Taniguchi had found that, while one inoculation of the rabbit with heterophile tissue produced sheep hemolysins, the repeated inoculation of rabbits with saline emulsion of guinea pig kidney produced ox hemolysins and that these ox hemolysins were complementary in degree to the sheep hemolysins produced. This suggested that the problem of heterophile hemolysins in human beings includes not only sheep hemolysins but ox hemolysins as well, and it was early apparent to us that ox hemolysins and ox cells should be used in the

TABLE IIB

CASE NO.	REMARKS	HEMOLYSINS												AGGLUTININS				BLOOD TYPE
		STRAIGHT LYTIC				TREATED WITH CELL SUSPENSION				TREATED WITH ALCOHOLIC ANTIGEN				STRAIGHT AGGLUTININ				
		SHEEP		OX		GUINEA PIG KIDNEY CELLS		OX KIDNEY CELLS		GUINEA PIG KIDNEY		OX KIDNEY		SHEEP		OX		
		Upper	*Lower*	*Upper*	*Lower*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Upper*	*Lower*	*Upper*	*Lower*	
13	Petit mal at infrequent intervals	C 1-8	90% 1-20	5% 1-2 50% 1-8 15% 1-16		O 1-2	O 1-2	C 1-2	O 1-2	AC 1 2	95% 1-2	C 1-2	C 1-2	++ 1-2	O 1-6	O 1-2	O 1-2	B
14	Grand mal at infrequent intervals Dementia praecox	Tr 1-20		Sl. Tr 1-20		O 1-2	O 1-2	O 1-2	O 1-2	35% 1-2 AC 99%	C 1-2 95% 1-2	AC 1-2 C 1-2	AC 1-2 C 1-2	O 1-2	O 1-2	O 1-2	O 1-2	A
15	Grand mal at infrequent intervals	O 1-2 O 1-2 O 1-2 O 1-2 O 1-2		O 1-2 O 1-2 O 1-2 O 1-2 O 1-2		O 1-2	O 1-2	O 1-2	O 1-2	O 1-2	O 1-2	10% 1-2	10% 1-2	+ 1-2		O 1-2		B
16	Grand mal almost daily. Died Nov. 30, 1936	C 1-16 C 1-2 C 1-2 C 1-2 C 1-2	Tr 1-96	C 1-12 C 1-2 C 1-2 C 1-2 C 1-2	30% 1-96	O 1-2 O 1-2	O 1-2 O 1-2	Tr 1-2 AC 1-2	Tr 1-2 O 1-2	O 1-2 O 1-2 C 1-2	Tr 1-2 O 1-2 C 1-2	O 1-2 Tr 1-2 C 1-2	Tr 1-2 Tr 1-2 C 1-2	++++ 1-4	± 1-20	++++ 1-2	O 1-12	A
17	Petit mal at frequent intervals	50% 1-2 O 1-2 Sl. Tr 1-2	O 1-8	O 1-2 O 1-2 O 1-2	O 1-2 O 1-2 O 1-2	O 1-2	O 1-2	Tr 1-2	O 1-2	Tr 1-2	20% 1-2	AC 1-2	50% 1-2	O 1-2	O 1-2	O 1-2	O 1-2	A

TABLE IIB—CONT'D

18	Grand mal at infrequent intervals (Note—Isophile hemolysins, Column 5)	C 1-6	20% 1-28	O 1-2	O 1-2	50% 1-2	O 1-2	C 1-2	O 1-2					++ 1-2		O 1-2		
19	Grand mal at infrequent intervals	C 1-2 C 1-2 C 1-2 C 1-2 C 1-2	Sl. Tr 1-8	C 1-2 C 1-2 C 1-2 C 1-2 AC 1-2	Sl. Tr 1-8	O 1-2 Tr 1-2 O 1-2	O 1-2 O 1-2 O 1-2	O 1-2 C 1-2 AC 1-2	O 1-2 O 1-2 O 1-2	C 1-2 O 1-2 40% 1-2 Tr 1-2 C 1-2	C 1-2 40% 1-2 C 1-2 C 1-2 C 1-2	C 1-2 Tr 1-2 C 1-2 C 1-2 C 1-2	C 1-2 70% 1-2 60% 1-2 C 1-2	++++ 1-2		++ 1-2		O
20	Grand mal at about 14-day intervals	C 1-4 C 1-2 C 1-2 C 1-2 C 1-2	Sl. Tr 1-80	50% 1-2 50% 1-2 C 1-2 50% 1-2 C 1-2	Sl. Tr 1-400	O 1-2	O 1-2	C 1-2	O 1-2	C 1-2	C 1-2	C 1-2	C 1-2	++++ 1-2	+ 1-8 O 1-2	O 1-2	O 1-2	O
21	Grand mal at infrequent intervals	Tr 1-4 O 1-2 Sl. Tr 1-2	Sl. Tr 1-8	O 1-2 O 1-2 O 1-2	O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 Tr 1-2	O 1-2 O 1-2	C 1-2 C 1-2	AC 1-2	C 1-2 C 1-2	AC 1-2	+ 1-4	± 1-6	O 1-2	O 1-2	B
22	Grand mal at infrequent intervals	C 1-12 C 1-2 C 1-2	40% 1-24	O 1-2 O 1-2 O 1-2	O 1-2	O 1-2	O 1-2	C 1-2	O 1-2	C 1-2 C 1-2 O 1-2	C 1-2 Tr 1-2 25% 1-2	C 1-2 C 1-2 50% 1-2	C 1-2 Tr 1-2 15% 1-2	++++ 1-4	± 1-16	O 1-2	O 1-2	O
23	Grand mal at infrequent intervals. Possibly hysteria	Tr 1-10 O	O 1-12	O 1-2 O 1-2	O 1-2	O 1-2	O 1-2	O 1-2	O 1-2	O 1-2 O 1-2	O 1-2	AC 1-2 O 1-2	O 1-2	O 1-2	O 1-2	O 1-2	O 1-2	A
24	Grand mal at long intervals (Note—Isophile hemolysins, Col. 5)	C 1-2 C 1-2 C 1-2		C 1-2 C 1-2 C 1-2		O 1-2 AC 1-2 AC 1-2	O 1-2 40% 1-2 60% 1-2	M50% 1-2 C 1-2 C 1-2	O 1-2 O 1-2 O 1-2	C 1-2 C 1-2	C 1-2 C 1-2	C 1-2 C 1-2	C 1-2 C 1-2	++++ 1-2		++ 1-2		A

lytic and heterophile complement fixation tests in order to avoid the complicating action of sheep hemolysins in human blood. We also found that guinea pig blood usually has a high titer of natural hemolysins for ox cells, that is, enough natural hemolysins to make it necessary to treat guinea pig complement with both sheep and ox cells before use in order to eliminate the factor of natural hemolysins in guinea pig complement.

The incidence of these immunologic factors in this group of epilepties is shown in Tables IIA, IIB, and IIC as follows:

Heat stabile sheep hemolysins, upper and lower levels.	Columns 1 and 2.
Heat stabile ox hemolysins, upper and lower levels.	Columns 3 and 4.
Adsorption tests, guinea pig kidney cells.	Columns 5 and 6.
Adsorption tests, ox kidney cells.	Columns 7 and 8.
Adsorption tests, alcoholic extract guinea pig kidney.	Columns 9 and 10.
Adsorption tests, alcoholic extract ox kidney.	Columns 11 and 12.
Agglutinins.	Columns 13 to 16.

CONTROLS

The blood of a group of nonepilepties was studied as a control. The sera were taken in the Clinical Laboratory from an unselected group of persons without regard to the reason for taking the blood. The group includes patients and employees. Among those showing no sheep or ox hemolysins were the following: employees, healthy—4; psychosis with organic brain disease, type undetermined—1; psychosis with cerebral arteriosclerosis—1; hysteria—1; psychosis with cerebral spinal syphilis—1; coronary sclerosis—1; paranoid state—1; neurasthenia—1; Huntington's chorea—1.

The findings in the remainder of the control group will be tabulated as follows:

TYPE	NUMBER PATIENTS	SHEEP HEMOLYSINS COMPLETE	SHEEP HEMOLYSINS NONE	OX HEMOLYSINS COMPLETE	OX HEMOLYSINS NONE
Dementia praecox	10	9	1	6	4
Highly allergic	2	2	0	2	0
Parkinson's syndrome	1	1	0	0	1
Cerebral arteriosclerosis	2	1	1	1	1
Alcoholic	1	1	0	1	0
General paralysis	1	1	0	1	0
Sciatica	1	1	0	0	1
Hyperthyroidism	1	1	0	0	1
Psoriasis in manic-depressive insanity	1	1	0	1	0
Multiple serum injections	1	1	0	1	0
Manic-depressive	1	1	0	0	1
General paralysis with Paget's disease	1	1	0	0	1

The complete analysis of this control group appears on Table IIIA.

DISCUSSION

A brief discussion of some of these patients is necessary to clarify the results. Patient No. 1 was discharged after being free from convulsions for one and a half years. At the time of discharge his blood showed a high titer of heat

stabile sheep hemolysins and a trace of heat stabile ox hemolysins. These sheep hemolysins proved to be both heterophile (column 7) and nonheterophile (column 5).

Two other patients, Nos. 9 and 24, showed both nonheterophile and heterophile heat stabile sheep hemolysins.

Case 5 is apparently recovering from convulsions. He formerly had severe grand mal attacks but had only two petit mal attacks in 1936. His blood showed no heat stabile hemolysins.

Case 11, from the history apparently a traumatic case, showed only small amounts of heat stabile sheep hemolysins.

Case 12, showed only traces of heat stabile sheep hemolysins on the direct lytic test, but moderate amounts of heat stabile sheep hemolysins after absorption with ox kidney (heterophile). This patient has had severe hay fever of the late type for many years.

Case 14, showing traces of heat stabile sheep hemolysins only, was diagnosed as paranoid praecox for ten years prior to any convulsive episode.

Case 15 is probably one of dementia praecox with a history of syphilis.

Case 31 is mute, shows snautzkrampf and is suicidal. The correct diagnosis is probably catatonic praecox.

Case 33 is possibly one of hysteria with gastric ulcer. His attacks may be due to spontaneous hypoglycemia.

Discussion of Results.—The results may be tabulated as follows:

Heat Stabile Sheep Hemolysins. Lytic Test.		
Almost complete or complete in higher dilutions		24 or 66 per cent (about)
Small amounts present		9 or 33 per cent (about)
Present in some amount		33 or 91 per cent (about)
None present		3 or 9 per cent (about)
Heat Stabile Ox Hemolysins. Lytic Test.		
Present in large amounts		15 or 41 per cent (about)
Present in small amounts		6 or 16 per cent (about)
Present in some amount		21 or 57 per cent (about)
None present		15 or 41 per cent (about)
After Adsorption With Guinea Pig Cells.		
Heat stabile sheep hemolysins.	Large amounts present	3 or 8 per cent (about)
	None present	33 or 91 per cent (about)
Heat stabile ox hemolysins.	Large amounts present	1 or 2 per cent (about)
	None present	35 or 97 per cent (about)
After Adsorption With Ox Kidney Cells.		
Heat stabile sheep hemolysins.	Large amounts present	25 or 70 per cent (about)
	None present	11 or 30 per cent (about)
Heat stabile ox hemolysins		0

Sheep Hemolysins.—Our study of sheep hemolysins in epileptic blood, based on over 100 tests done on 36 patients, therefore, showed that about 90 per cent of the group had heat stabile sheep hemolysins. One should, however, consider the natural incidence of sheep hemolysins in *human* serum. Kolmer and Rule[22] found about 95 per cent of fresh unheated human serum to contain hemolysins for sheep's corpuscles. Our results also agree with Kolmer in that these sheep hemolysins are heat stabile (thirty minutes at 56°). We found these sheep hemolysins to be of two kinds: heterophile and nonheterophile.

TABLE IIC

CASE NO.	REMARKS	HEMOLYSINS												AGGLUTININS				BLOOD TYPE
		STRAIGHT LYTIC				TREATED WITH CELL SUSPENSION				TREATED WITH ALCOHOLIC ANTIGEN				STRAIGHT AGGLUTININ				
		SHEEP		OX		GUINEA PIG KIDNEY CELLS		OX KIDNEY CELLS		GUINEA PIG KIDNEY		OX KIDNEY		SHEEP		OX		
		Upper	*Lower*	*Upper*	*Lower*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Upper*	*Lower*	*Upper*	*Lower*	
25	Petit mal weekly	Sl. Tr 1-3 O 1-2 O 1-2	O 1-4	O 1-2 O 1-2 O 1-2	O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	50% 1-2	O 1-2 60% 1-2	50% 1-2	O 1-2	O 1-2	O 1-2	O 1-2	A
26	Grand mal at infrequent intervals	Sl. Tr 1-6 O 1-2 O 1-2	O 1-8	O 1-2 M 20% 15% 1-2	O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	AC 1-2 95% 1-2	85% 1-2	AC 1-2 AC 1-2	C 1-2	+ 1-2	O 1-6	O 1-2	O 1-2	O
27	Alcoholic	50% 1-2 75% 1-2	5% 1-24	O 1-2	O 1-2	O 1-2	O 1-2	AC 1-2	O 1-2	M 85% 1-2 O 1-2	15% 1-2 O 1-2	C 1-2 O 1-2	15% 1-2 O 1-2	++++ 1-3	± 1-12	O 1-2	O 1-2	B
28	Grand mal at infrequent intervals	C 1-16 C 1-2 C 1-2	60% 1-96	Tr 1-2 O 1-2 O 1-2	Tr 1-96	O 1-2	O 1-2	C 1-2	O 1-2	C 1-2	O 1-2	C 1-2	O 1-2	++++ 1-2	± 1-10	O 1-2	O 1-2	B
29	Grand mal at infrequent intervals	C 1-2 Tr 1-2 5% 1-2 O 1-2 Tr 1-2	Tr 1-28 Sl. Tr 1-8	C 1-2 C 1-2 50% 1-2 AC 1-2 75% 1-2	40% 1-96 O 1-8	O 1-2 O 1-2	Anti-comp. O 1-2	C 1-2 O 1-2	O 1-2 O 1-2	85% 1-2	AC98% 1-2	90% 1-2	95% 1-2	O 1-2	O 1-2	O 1-2	O 1-2	O
30	Grand mal at infrequent intervals	M 25% 1-2 AC 1-2 C 1-2	O 1-2	O 1-2 O 1-2 O 1-2	O 1-2	O 1-2	O 1-2	90% 1-2	O 1-2	O 1-2 65% 1-2	O 1-2 AC 1-2	Tr 1-2 90% 1-2	O 1-2 75% 1-2	++ 1-2		± 1-2		A

TABLE IIC—CONT'D

31	Grand mal infrequent intervals Catatonic praecox; mute	C 1-6 C 1-2 C 1-2 AC 1-8 90% 1-2		75% 1-2 Tr 1-2 AC 1-2 Tr 1-2 AC 1-2	O 1-6	O 1-2	O 1-2	C 1-2	O 1-2	90% 1-2	AC 1-2	C 1-2	C 1-2	+ 1-2		O 1-2		O
32	Grand mal weekly	15% 1-2 O 1-2 O 1-2 O 1-2 O 1-2		Sl. Tr 1-2 O 1-2 O 1-2 O 1-2 O 1-2		O 1-2	O 1-2	25% 1-2	O 1-2	50% 1-2	50% 1-2	75% 1-2	25% 1-2	± 1-2		O 1-2		O
33	Petit mal at infrequent intervals Possibly hysteria	Sl. Tr 1-2 O 1-2 O 1-2 O 1-2 O 1-2		O 1-2 O 1-2 O 1-2 O 1-2 O 1-2		O 1-2	O 1-2	O 1-2	O 1-2	O 1-2	Tr 1-2	10% 1-2	Tr 1-2	O 1-2		O 1-2		O
34	Grand mal at infrequent intervals	C 1-4 AC98% 1-2 M 30% 1-2	Tr 1-40	O 1-2 O 1-2 O 1-2	O 1-2 O 1-2 O 1-2	O 1-2	O 1-2	AC 1-2	O 1-2	Sl. Tr 1-2	O 1-2	50% 1-2	Sl. Tr 1-2	± 1-2	O 1-4	O 1-2	O 1-2	A
35	Grand mal at infrequent intervals	C 1-12 C 1-2 C 1-2	90% 1-24	60% 1-2 C 1-2 AC 1-2	Tr 1-4	O 1-2 Tr 1-2	O 1-2 O 1-2	20% 1-2 C 1-2	O 1-2 O 1-2	O 1-2 90% 1-2	90% 1-2	C 1-2 40% 1-2	95% 1-2	++++ 1-6	± 1-12	O 1-2	O 1-2	O
36	Grand mal at infrequent intervals	Tr 1-2 O 1-2 O 1-2 O 1-2 O 1-2		65% 1-2 O 1-2 O 1-2 O 1-2 O 1-2		O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2 O 1-2	O 1-2	50% 1-2	90% 1-2	50% 1-2	O 1-2		O 1-2		O

Ox Hemolysins.—Our study of ox hemolysins in this same group showed that there is a high titer of ox hemolysins in the blood of many epilepties (about 41 per cent), and that some ox hemolysins are present in about 57 per cent. This also should be considered in connection with the normal incidence of ox hemolysins. Kolmer (loc. cit.) found about 78 per cent of unheated human sera to have hemolysins for ox corpuscles and about 47 per cent of heated sera to contain anti-ox hemolysins. Agreeing with Kolmer, we found that the natural ox hemolysins occurring in the blood of epileptic, dementia praecox, and sensitized persons are heat stabile (see also later adsorption tests with guinea pig kidney).

Our study of the ox hemolysins was still more interesting in view of the work of Taniguchi (loc. cit.) who had shown that one injection of guinea pig kidney tissue into the rabbit resulted in the production of sheep hemolysins, whereas multiple injections produced ox hemolysins. One would, therefore, expect heterophile ox hemolysins to occur in some cases where heterophile sheep hemolysins were absent. Reference to the tabulated results will show that this actually occurs (Cases 26 and 36). We also found that these ox hemolysins differed from the heat labile natural ox hemolysins in that they are of two kinds: (a) heterophile and (b) nonheterophile. These hemolysins are quite different from those normally found in the human blood: they are adsorbed by guinea pig kidney cells, and whereas the ox hemolysins in normal blood disappear quite rapidly on standing in the ice box (even over night), ox hemolysins in the blood of epilepties, dementia praecox, and highly sensitized persons remain stabile as long as do the sheep hemolysins.

We may summarize these results by saying that almost all cases which could reasonably be called "idiopathic epilepsy" contained sheep or ox hemolysins which were, in the usual meaning of the term, heat stabile.

Adsorption Lytic Tests.—(a) Adsorption with guinea pig kidney cells. (1) Sheep Hemolysins: The sheep hemolysins occurring in epileptic blood were adsorbed with saline emulsion of guinea pig kidney cells in all cases with the exception of four (one a patient who has recovered from convulsions and three epilepties who have some hemolysins which were adsorbed and others which were not adsorbed by guinea pig kidney cells), showing that there are two kinds of sheep hemolysins: (a) those which are adsorbed with cell suspensions of guinea pig kidney, and (b) those which are not, the former being heterophile and the latter not heterophile. (2) Ox Hemolysins: We were able to adsorb ox hemolysins by guinea pig kidney cells in every case except one (No. 24).

(b) Adsorption with ox kidney cells. (1) Sheep Hemolysins: With the exception of one case (No. 17), we were *unable* to adsorb the sheep hemolysins with suspensions of ox kidney cells whenever they occurred in a high titer. In fact the presence of sheep hemolysins after treatment with ox kidney cell suspension served as an excellent check of their presence in the lytic tests. In one case (No. 12) the sheep hemolysins were present in high titer after adsorption with ox kidney cells, whereas there was only a trace in straight lytic tests. (2) We have fixed ox hemolysins in every case with a suspension of ox kidney.

TABLE IIIA

CASE NO.	REMARKS	HEMOLYSINS												AGGLUTININS			
		STRAIGHT LYTIC				TREATED WITH CELL SUSPENSION				TREATED WITH ALCOHOLIC ANTIGEN				STRAIGHT AGGLUTININ			
		SHEEP		OX		GUINEA PIG KIDNEY CELLS		OX KIDNEY CELLS		GUINEA PIG KIDNEY		OX KIDNEY		SHEEP		OX	
		Upper	*Lower*	*Upper*	*Lower*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Sheep*	*Ox*	*Upper*	*Lower*	*Upper*	*Lower*
	Dementia praecox (8 cases)	C		C		O	O	C	O	C	C	C	C				
	Highly allergic (2 cases)	C C		C C		C O	O	C C	O	C O	C O	C O	C O				
	Parkinsonian syndrome	C		O		—	—	—	—	C	O	C	C				
	Psychosis with cerebral arteriosclerosis	C		C		—	—	—	—	—	—	—	—				
	Psychosis—alcoholic	C		C		O	O	C	O	C	C	C	C				
	General paralysis	C		C		O	O	C	O	C	O	C	O				
	Sciatica	C		O		O		C									
	Hyperthyroidism	C		O		—	—	—	—	—	—	—	—				
	Manic-depressive insanity with psoriasis	C		C		O	O	C	O	C	C	C	C				
	Multiple serum injections	C		C													
	General paralysis with Paget's disease	C		O		—	—	—	—	—	—	—	—				

CONTROLS

When the incidence of heat stabile hemolysins in the control group was investigated, the occurrence of heat stabile hemolysins in the blood of epilepties was given additional importance. With the exception of four attendants, who were added to the control group with the expectation that they would not show heterophile antibodies, no selection had been made in these controls. These controls proved, however, to be of two types: (a) those persons who were not suffering from well-marked disease at the time the blood was taken or who had not been given horse serum during the past few years; these cases, together with the four healthy employees and other persons who were not heavily sensitized, did not show heat stabile hemolysins. This first group of controls stands in marked contrast with the second group, *a large percentage of which were allergic persons of all types* (asthma, hay fever, urticaria, alcoholism, and multiple serum injections), *dementia praecox, manic-depressive insanity with psoriasis, all of whom were found to have heat stabile sheep and ox hemolysins.* Further, these hemolysins were proved to be heterophile by cross adsorption tests and also by complement fixation.

Therefore, while we had shown the presence of heterophile sensitization in epilepsy, we were forced to abandon the hope that it would be found only in epilepsy and therefore specific to this condition.

The agglutinins will be considered in a separate article and have been included in this tabulation as a matter of general interest. In some cases they have helped to identify the sheep hemolysins as being heterophile.

In addition, heterophile complement fixation tests were done. These also will be discussed in a separate article. Suffice to say at this time that in three out of four cases where the hemolysins were present in very small quantities, the heterophile nature of these hemolysins was confirmed by complement fixation.

More than special interest attaches, however, to the results of treatment of the blood of these epileptic patients with the alcoholic extract of guinea pig and ox kidney. These results also will be considered in a separate article in connection with coctoprecipitins and the conglutinin test. Briefly, these adsorption tests not only identified the heterophile hemolysins but uncovered a phenomenon of great interest: our attempts to fix *sheep hemolysins* with alcoholic extract of guinea pig kidney or ox kidney resulted in the *lighting up of dormant or "masked" hemolysins so that the hemolysis was actually increased.* This stands in sharp contrast to the work of Taniguchi on rabbits. We were fully aware of the fact that the use of alcoholic tissue extracts as antigen at times gives very unreliable results. We were able, however, to control our work with these antigens so carefully that we were led to believe that we were possibly dealing with some lytic substance in the blood heretofore unrecognized; but the probable explanation lies in the dormant or "masked" hemolysin in the epileptic blood contained in loose combination with heterophile antigen. This coexistence of antigen and antibody in the blood in loose combination may be very important in connection with epilepsy.

Nor have we been able to adsorb the ox hemolysins with alcoholic extract of guinea pig kidney or of ox kidney. On the contrary, blood so treated shows an "unmasking" or increase of the ox hemolysins present. However, as stated above, the ox hemolysins are adsorbed by both heterophile and nonheterophile kidney cell suspensions and, therefore, while we believe that they are heterophile in nature, they are not identical with those *sheep hemolysins* which are fixed by heterophile tissue only. This phenomenon has not been heretofore explained and requires further investigation.

Finally we come to a phenomenon of the greatest importance. All of the above observations were made before it was known that the occurrence of a convulsion may change the hemolysin content of the blood. By referring to column 1 of Tables IIA, IIB, and IIC, it will be seen that many cases show bloods with a sufficiently high titer of sheep hemolysins to give complete or almost complete hemolysis on one test and then on a subsequent test show little or no sheep hemolysins. This was very confusing until it was realized that the variation was due to the occurrence of a convulsion, which knowledge modified the whole subsequent plan of the research. It is mentioned at this time merely to explain the irregularity in the occurrence of heterophile sheep hemolysins and will be discussed in a later article.

SUMMARY

This study of heterophile hemolysins, investigating the hypothesis that they exert definite influence in many clinical forms of nervous and mental disease, constantly kept in mind the philosophical point of view outlined as the body/mind concept.

A brief history of the development of the concept of heterophile sensitization was shown by abstracting the findings of other writers on heterophile sensitization and also sensitization as a cause of epilepsy.

Following this, a brief report of our preliminary work on heterophile sensitization in a small group of epileptics was outlined and the results charted.

After a detailed description of the methods and material used in the research which was authorized to investigate this hypothesis, our findings with reference to the occurrence of heterophile hemolysins, both sheep and ox; adsorption with saline suspensions of guinea pig and ox kidney cells; and, finally, adsorption with alcoholic extracts of guinea pig and ox kidney were given.

The results in control groups of healthy and sensitized persons were added for comparison. It was shown that while epileptics are persons heavily sensitized with heterophile antigen, the same sensitization also occurs in other allergies with a difference in mechanism.

This study briefly referred to the heterophile precipitins and agglutinins, heterophile complement fixation, the lighting up of "masked" hemolysins, the coexistence of antigen and antibody in loose combination, and finally to the fact that the titer of heterophile hemolysins is altered at the time convulsions occur. These subjects will be discussed in subsequent studies.

REFERENCES

1. Lazell, E. W.: The Anatomy of Emotion, New York, 1929, D. Appleton-Century Co.
2. Lazell, E. W.: A Clinical Study of the Emotional Factors Which Precipitate the Epileptiform Convulsion. To be published.
3. Lazell, E. W.: Sensitization in the Convulsive States With Special Reference to Heterophile Antigen. Submitted for publication May 13, 1935.
4. Lazell, E. W., and Prince, L. H.: A Study of the Causative Factors of Dementia Praecox; the Influence of the Blood and Serum on Embryological Cells; Preliminary Communication, U. S. Vet. Bur. M. Bull. **5**: 40, 1929.
5. Lazell, E. W.: The Forssman or Heterophile Antigen, M. Bull. Vet. Admin. **8**: 477, 1932.
6. Taniguchi, T.: Studies in Heterophile Antigen and Antibody, J. Path. & Bact. **24**: 456, 1921.
7. Bailey, G. H.: Accessory Etiologic Factors of Respiratory Infection on Rabbits, Am. J. Hyg. **9**: 192, 1929.
8. Davidsohn, I.: Heterophile Antigen in Human Blood, Arch. Path. **6**: 632, 1928.
9. Davidsohn, I., and Ramsdell, S. G.: Horse Serum as a Heterophile Antigen, J. Immunol. **17**: 365, 1929.
10. Donnally, H. H.: The Question of the Elimination of Foreign Protein (Egg-White) in Woman's Milk, J. Immunol. **19**: 15, 1930.
11. Holford, F. E.: The Placental Transmission of Foreign Proteins in Rabbits, J. Immunol. **19**: 177, 1930.
12. Butt, E. M., and Foord, A. G.: The Heterophile Antibody Reaction in the Diagnosis of Infectious Mononucleosis, J. Lab. & Clin. Med. **20**: 538, 1935.
13. Paul, J. R., and Bunnell, W. W.: The Presence of Heterophile Antibodies in Infectious Mononucleosis, Am. J. M. Sc. **183**: 90, 1932.
14. Forman, J.: Atopy as a Cause of Epilepsy, Arch. Neurol. & Psychiat. **32**: 517, 1934.
15. Howell, L. P.: Epilepsy and Protein Sensitization, Ohio State M. J. **19**: 660, 1923.
16. Rackemann, F. M.: Allergy; Review of Current Literature, Arch. Int. Med. **55**: 141, 1934.
17. Rowe, Albert H.: Clinical Allergy, Manifestations, Diagnosis and Treatment, Philadelphia, 1937, Lea and Febiger.
18. Kolmer, John A.: Infection, Immunity and Biologic Therapy, ed. 3, Philadelphia, 1925, W. B. Saunders Co., p. 658.
19. Kahn, R. S.: Tissue Immunity, Springfield, 1936, C. C. Thomas.
20. Taniguchi, T.: Studies on Heterophile Antigen and Antibody, J. Path. & Bact. **24**: 217, 1921.
21. Kolmer, J. A.: Infection, Immunity and Biologic Therapy, ed. 3, Philadelphia, 1925, W. B. Saunders Co., p. 395.
22. Kolmer, J. A., and Rule, A.: Studies in Standardization of Wassermann Reaction; Influence of Natural Antisheep Hemolysin in Human Sera Upon Wassermann Reaction, Am. J. Syph. **4**: 135, 1920.

LABORATORY METHODS

A COMPARATIVE STUDY OF SELECTIVE MEDIA FOR THE ISOLATION OF TYPHOID BACILLI FROM STOOL SPECIMENS*

A. A. Hajna, M.S., and C. A. Perry, Sc.D., Baltimore, Md.

DURING recent years, several new selective media for the isolation of typhoid bacilli from stool specimens have appeared in the literature. The majority of these were devised to supplement, or even to supplant, such widely used media as Endo's sulfite fuchsin dye agar, Holt-Harris and Teague's eosin methylene blue agar medium, etc. The present investigation was undertaken to study the relative merits of some of these media, and to determine what combination was most practical and efficient for the routine isolation of typhoid bacilli from stool specimens.

PREPARATION OF MEDIA

Most of the selective media used in this study are more or less inhibitory. Particular care was used to follow explicitly, directions of the authors for the preparation of various media. Certain modifications of the Wilson and Blair procedure for the preparation of their medium have been found desirable. The method of preparation is given elsewhere in this paper.

The following media have been chosen for this study.

Solid Media.—A. A medium that neither suppresses nor inhibits organisms, but serves to differentiate colonies on the basis of fermentation of lactose as indicated by a change of color in the indicator used: Bromeresol purple lactose agar (Chesney, 1922).

B. Media that suppress or inhibit the Gram-positive organisms but not the Gram-negative group and on which there is a sharp differentiation by means of appropriate indicators between lactose fermenting and non-lactose fermenting colonies: Endo (1904); Endo (Robinson and Rettger, 1916); eosin methylene blue (Holt-Harris and Teague, 1916); MacConkey (1905); and desoxycholate (Leifson, 1935).

C. Media that contain chemicals designed to delay or completely suppress the development of Gram-negative bacteria of the colon and other groups and to allow a vigorous growth of *E. typhosa:* Eosin brilliant green (Teague and Clurman, 1916); brilliant green (Krumwiede and others, 1916); desoxycholate citrate (Leifson, 1936); and bismuth sulfite brilliant green (Wilson and Blair, 1927 and 1931).

*From the Bureau of Bacteriology, Maryland State Department of Health, Baltimore.
Received for publication, December 2, 1937.

Since the preparation of bismuth sulfite brilliant green agar medium of Wilson and Blair (1927 and 1931) differs here in some respects, such as titration of base and the preparation of certain chemicals, from the original formula, it is thought worth while to describe it.

Preparation of Nutrient Agar Base

To 1,000 c.c. of hot distilled water are added:

Bacto-beef extract	5 gm.
Bacto-peptone	10 gm.
Bacto-glucose	5 gm.
Bacto-agar	30 gm.

The above ingredients are dissolved by heating in the Arnold sterilizer. No titration or filtration is necessary.

To each 1,000 c.c. of hot melted nutrient agar are added:

70 c.c. of sulfite bismuth phosphate iron solution
3 to 5 c.c. of a 1 per cent aqueous solution of brilliant green dye (preferably Grübler's)

Autoclave at 15 pounds pressure for not more than ten minutes. Do not pour into Petri dishes in direct sunlight, as sunlight brings out the brilliant green dye. If plates showing light green color are used, typical typhoid colony characteristics will be absent.

Do not leave plates at room temperature longer than necessary to solidify and dry the surface before streaking and incubating them. The plated medium will keep for three or four days, however, if refrigerated immediately after solidifying.

The preparation of stock sulfite bismuth phosphate iron solution is as follows:

a. 20 per cent aqueous solution anhydrous sodium sulfite (Eastman)	100.0 c.c.
b. Ammoniacal solution of bismuth citrate	50.0 c.c.
c. Anhydrous dibasic sodium phosphate (Merck's or Baker's)	10.5 gm.
d. 8 per cent aqueous solution (acidified with two drops of conc. HCl) ferrous sulfate crystals (Merck's or Baker's)	10.0 c.c.

Boil gently until slate gray color appears. This forms a stock solution which can be kept at room temperature for months, if kept sealed with a rubber stopper.

The ammoniacal solution of bismuth citrate is prepared as follows:

Mix 6 gm. of bismuth citrate (Merck) with 10 to 20 c.c. of distilled water in a 250 c.c. Erlenmeyer flask, and then add approximately 3 c.c. of very strong ammonia. The mixture should be agitated continuously while adding ammonia until the solution becomes almost clear. The volume is then made up to 50 c.c. with distilled water.

Liquid Media.—A. *Preservative Medium:* Perry, Hajna, and Robinson, in 1933, made a comparative study (unpublished work) of preservative media for delayed examination of stool specimens, i.e., in transit from patient or carrier to the laboratory. Of the five different media, lithium chloride bile, brilliant green bile, plain bile, glycerol salt, and lithium chloride glycerol salt, the plain bile was finally chosen. It was chosen for routine examination and for research in this work, because it not only has no harmful effect on typhoid bacilli, but under ordinary circumstances, is a good bacteriostatic medium for the Gram-positive bacteria. Bile also temporarily arrests the growth of Gram-negative nonpathogens.

B. *Fluid Enrichment Media:* For the enrichment of typhoid bacilli from stool specimens: tetrathionate broth (Müller, 1923); tetrathionate bile brilliant green broth (Kauffmann, 1930-31); brilliant green Esbach's reagent broth (Ruys, 1934); and selenite broth (Leifson, 1936).

EXPERIMENTAL

Solid Media.—Growth of Pure Cultures of E. typhosa and Esch. coli on Various Media: A loopful (3 mm. in diameter) of twenty-four-hour peptone broth cultures of *E. typhosa* and *Esch. coli* were spread on the surfaces of agar media under investigation. The growth of the two organisms on the media was compared with the growth of the same cultures on bromeresol purple (BCP) lactose agar, which is a nonselective medium. The results are summarized in Table I.

TABLE I

GROWTH OF *E. typhosa* AND *Esch. coli* ON VARIOUS SOLID MEDIA

MEDIUM	NO. OF STRAINS TESTED	*E. typhosa*	NO. OF STRAINS TESTED	*Esch. coli*
BCP Lactose (Chesney)	56	usually 4+	56	usually 4+
Endo (original)	10	usually 4+	10	usually 4+*
Endo (Robinson and Rettger)	10	usually 4+	10	usually 4+*
EMB (Difco formula)	56	+ to 4+	56	3+ to 4+
MacConkey (Difco formula)	10	usually 4+	10	4+
Desoxycholate (Leifson)	56	usually 4+	56	4+
Eosin brilliant green (Teague and Clurman)	10	2+ to 4+	10	+ to 2+
Brilliant green (Krumwiede)	10	+ to 4+	10	0 to 2+
Desoxycholate citrate (Leifson)	56	2+ to 4+	56	usually 0, occasionally +
Wilson and Blair (Modified)	136	usually 3+ to 4+	136	usually 0, occasionally +

*Agar often reddened.

0 = no growth. + = scanty. 2+ = fair. 3+ = moderate. 4+ = heavy.

Comparative tests of the ten agar media made with suspension of pure cultures of *Esch. coli* showed that there is no inhibition of such strains by the two Endo media, the eosin methylene blue, MacConkey and desoxycholate media. Marked restrictions of *Esch coli* were observed on the eosin brilliant green and brilliant green media, while the almost complete restriction of any growth of *Esch. coli* was found on the desoxycholate citrate and the bismuth sulfite agar media. On these two latter media, the growth of *E. typhosa* is usually not restricted. Very infrequently, a strain is found which, on primary cultivation (especially from blood), is somewhat inhibited. There is not much difference in the amount of growth of *E. typhosa* and *Esch. coli* on Endo, Endo (Robinson and Rettger), MacConkey and desoxycholate media. Typhoid cultures grew better on the eosin brilliant green medium than on Krumwiede's brilliant green medium. Great variation in the amount of growth was noted on the eosin methylene blue medium.

Isolation of E. typhosa From Artificial Mixtures of E. typhosa and Esch. coli: Various dilutions of twenty-four-hour peptone broth cultures of *E. typhosa* were mixed with constant quantities of broth cultures of *Esch. coli.* The mixed cultures were then incubated at 37° C. for three hours. A loopful was spread on the surface of the media. Results are tabulated in Table II.

TABLE II

EFFICIENCY OF VARIOUS AGAR MEDIA IN THE RE-ISOLATION OF *E. typhosa* IN VARIOUS DILUTIONS WITH CONSTANT QUANTITY OF *Esch. coli*

MEDIUM	EXPERIMENTAL—3 STRAINS OF *E. typhosa* AND *Esch. coli* USED			
	1 c.c. *E. typhosa* + 1 c.c. *Esch. coli*	1 c.c. of 1-10 *E. typhosa* + 1 c.c. *Esch. coli*	1 c.c. of 1-100 *E. typhosa* + 1 c.c. *Esch. coli*	1 c.c. of 1-1000 *E. typhosa* + 1 c.c. *Esch. coli*
Bromcresol purple	*Esch. coli* predominant; occasionally few *E. typhosa*	All *Esch. coli*	All *Esch. coli*	All *Esch. coli*
Endo	*Esch. coli* predominant; in well-isolated cases, many *E. typhosa*	*Esch. coli* predominant, few of *E. typhosa* marked by red color	All *Esch. coli*	All *Esch. coli*
Endo (R + R)	*Esch. coli* predominant; in well-isolated cases, many *E. typhosa*	*Esch. coli* predominant, few of *E. typhosa* marked by red color	All *Esch. coli*	All *Esch. coli*
Eosin methylene blue	*Esch. coli* predominant; in well-isolated cases, many *E. typhosa*	*Esch. coli* predominant, fewer of *E. typhosa* than on Endo	All *Esch. coli*	All *Esch. coli*
MacConkey	*Esch. coli* predominant; in well-isolated cases, many *E. typhosa*	*Esch. coli* predominant, number of *E. typhosa* same as on Endo	Very few *E. typhosa*, nearly all *Esch. coli*	All *Esch. coli*
Desoxycholate	*Esch. coli* predominant; in well-isolated cases, many *E. typhosa*	*Esch. coli* predominant, number of *E. typhosa* same as on Endo	Very few *E. typhosa*, nearly all *Esch. coli*	All *Esch. coli*
Eosin brilliant green	Equal numbers of colonies of *Esch. coli* and *E. typhosa*	Equal numbers of *Esch. coli* and *E. typhosa*	*Esch. coli* predominant, numerous *E. typhosa*	*Esch. coli* predominant, very few *E. typhosa*
Brilliant green	Equal numbers of colonies of *Esch. coli* and *E. typhosa*	Equal numbers of *Esch. coli* and *E. typhosa*	*Esch. coli* predominant, numerous *E. typhosa*	*Esch. coli* predominant, very few *E. typhosa*
Desoxycholate citrate	Very few *Esch. coli*, *E. typhosa* predominant	Very few *Esch. coli*, *E. typhosa* predominant	Very few *Esch. coli*, numerous *E. typhosa*	Very few *Esch. coli*, few *E. typhosa*
Wilson and Blair	No *Esch. coli*, all *E. typhosa*	No *Esch. coli*, all *E. typhosa*	No *Esch. coli*, all *E. typhosa*	No *Esch. coli*, all *E. typhosa*

E. typhosa cannot be detected when present along with large numbers of *Esch. coli,* when media such as Endo, MacConkey, desoxycholate and eosin methylene blue agar are used. If there is some chemical, such as brilliant green, in the medium which restricts the growth of *Esch. coli, E. typhosa* grows. Desoxycholate citrate and bismuth sulfite proved to be the best media.

Isolation of E. typhosa From Twenty-Five Stool Specimens: Specimens were plated on all the media listed in Table II at the same time and subjected to the same conditions and time of incubation. In each instance, colonies were picked and confirmed culturally.

TABLE III

EFFICIENCY OF SELECTIVE AGAR ON THE DIRECT ISOLATION OF *E. typhosa* FROM POSITIVE STOOL SPECIMENS

MEDIUM	NUMBER POSITIVE	RESULTS—USING 25 POSITIVE SPECIMENS
Bromeresol purple	2	Often overgrown with lactose fermenting bacilli and proteus
Endo	5	Often overgrown with lactose fermenting bacilli; typhoid colonies often masked by reddened medium
Endo (R + R)	6	Often overgrown with lactose fermenting bacilli; typhoid colonies often masked by reddened medium
Eosin methylene blue	6	*Esch. coli* and *A. aerogenes* predominant
MacConkey	4	Lactose fermenting bacilli predominant
Desoxycholate	4	Lactose fermenting bacilli predominant
Eosin brilliant green	12	*Esch. coli* predominant
Brilliant green	12	*Esch. coli* predominant
Desoxycholate citrate	18	Growth of *Esch. coli* often inhibited, but other bacilli not often inhibited
Wilson and Blair	22	Often the plates show pure cultures of *E. typhosa;* rarely *Esch. coli* encountered; *A. aerogenes* more frequently encountered

NOTE: Three specimens, previously positive for *E. typhosa* (iced two days), failed to yield *E. typhosa* on any of the plates.

The greatest number of confirmations of the presence of *E. typhosa* was obtained on Wilson and Blair's medium (a total of 22). Eighteen positive results were observed on the desoxycholate citrate medium. Results on eosin brilliant green and brilliant green media were about the same (12 positives). The rest of the media yielded fewer *E. typhosa. E. typhosa* could be isolated from only 2 of the 25 specimens when bromeresol purple lactose agar was used.

Fluid Enrichment Media.—The fluid enrichment media were tested with the main object of finding the one in which *E. typhosa* multiplies more rapidly than the common nonpathogenic fecal bacteria. The six plate media, already described, were used for isolation from each of the liquid media to determine which combination of liquid and solid media was particularly advantageous for isolating the typhoid bacillus.

Of 28 specimens, *E. typhosa* was isolated from 16, and *S. schottmülleri* from 3. Seventeen of these 19 "positives" were found on plating directly from the bile preservative medium.* The greatest number (18), however, were

*Stool specimens are collected on swabs which are placed in rubber stoppered test tubes containing 5 c.c. of beef bile adjusted to pH 6.5.

found after enrichment in Leifson's selenite "F" medium, 14 from Müller's tetrathionate medium, 15 from Kauffmann's tetrathionate bile brilliant green medium, and 12 from Ruys' brilliant green Esbach's reagent medium. The three specimens positive for *S. schottmülleri* were positive in all liquid media.

Wilson and Blair's plating medium was superior to all others tested as more specimens were positive on this medium regardless of what enrichment medium was first used.

TABLE IV

COMPARATIVE VALUE OF FLUID MEDIA IN THE ENRICHMENT OF *E. typhosa* IN 28 SPECIMENS AND THE COMPARATIVE EFFICIENCY OF AGAR MEDIA IN THE ISOLATION FROM FLUID MEDIA

TOTAL POSITIVE SPECIMENS	LIQUID ENRICHMENT MEDIA	POSITIVE SPECIMENS MISSED	NUMBER OF POSITIVE SPECIMENS ON VARIOUS PLATING MEDIA						
			TOTAL CONFIRMATIONS	BCP	EMB	MAC C.	DES.	DES. CIT.	W. & B.
19	Bile*	2	14 Ty	2	4	2	2	7	14
			3 "B"	1	1	1	1	3	3
	Selenite "F"	1	15 Ty	4	8	8	9	9	15
			3 "B"	1	1	3	3	3	3
	Müller's	5	11 Ty	2	3	5	5	6	11
			3 "B"	1	1	1	1	3	3
	Kauffmann's	4	12 Ty	4	6	6	6	11	12
			3 "B"	1	1	1	1	3	3
	Ruys'	7	9 Ty	1	1	1	1	3	9
			3 "B"	0	0	0	0	3	3

*Specimen received in this medium, then 1 c.c. transferred to each of the other fluid media.

NOTE: Nine specimens negative in all fluid media.

W & B = Wilson and Blair's bismuth sulfite
Des. = Desoxycholate
Des. cit. = Desoxycholate citrate
EMB = Holt-Harris and Teague's eosin methylene blue
BCP = Chesney's bromcresol purple lactose
MacC. = MacConkey
Ty = typhoid
"B" = paratyphoid "B"

Evaluation of the Combined Use of Selenite Broth and Bismuth Sulfite Agar in Routine Stool Examinations.—In order to ascertain the value of selenite broth and bismuth sulfite agar in routine stool examinations more definitely, inoculations were made on six other plating media simultaneously with inoculations on bismuth sulfite medium. Since all media were not inoculated with the same specimens of stool, comparisons have been tabulated between Wilson and Blair's medium and one other medium on the same stool specimens, and the per cent of positive isolations noted. The ratio of these two per cents is taken as the index of the comparative value of the two media.

Wilson and Blair's medium yielded the highest percentage of positive results, both before and after enrichment. Leifson's desoxycholate citrate medium yielded the next highest percentage of positive isolations; the ratios being 0.67 and 0.85, respectively. The indices for Leifson's desoxycholate medium were 0.46 and 0.78; for Holt-Harris and Teague's eosin methylene blue medium 0.42 and 0.78; while Chesney's bromcresol purple lactose agar, the indices were 0.18 and 0.53; and for MacConkey's medium, 0.16 and 0.33.

In one or two instances, strains of *E. typhosa* have been encountered which give colorless colonies on the bismuth sulfite medium. This occurs rarely. Such colonies can be recognized as suspicious, by one with experience.

Typhoid colonies of the rough phase occasionally appear on this medium. They may be identified by the irregular contour of the colony, with raised center. The dusky halo may not be present, but the characteristic black center with colorless periphery is sufficient means of detection of such strains.

It is to be noted that the chances for isolating *E. typhosa* from stool specimens are greatly increased after enrichment in the selenite liquid medium of Leifson. Leifson has pointed out the principle by which the growth of the majority of fecal bacteria is held in check during the first eighteen hours of incubation at 37° C., while *E. typhosa* increases rapidly. The value of this enrichment medium is indirectly proportional to the selective nature of the plating medium used, but in all instances a significant increase of specimens yielding *E. typhosa* has been found after enrichment.

TABLE V

PERCENTAGE OF STOOL SPECIMENS POSITIVE FOR *Eberthella typhosa* ON WILSON AND BLAIR'S BISMUTH SULFITE MEDIUM AS COMPARED WITH OTHER PLATING MEDIA AND INCREASES DUE TO ENRICHMENT IN LEIFSON'S SELENITE "F" BROTH

(Compare Percentages Horizontally)

WILSON AND BLAIR VERSUS	NO. SPECIMENS COMPARED	BILE PRESERVATIVE			SELENITE ENRICHMENT		
		W. AND B.	MEDIUM COMPARED	RATIO	W. AND B.	MEDIUM COMPARED	RATIO
Desoxycholate citrate	347	17.3	11.5	0.67	18.7	15.9	0.85
Desoxycholate	45	53.4	24.5	0.46	60.0	46.7	0.78
Eosin methylene blue	150	31.4	13.3	0.42	34.6	27.4	0.78
Bromeresol purple	895	11.5	2.1	0.18	14.6	8.7	0.53
Brilliant green	102	7.8	0	0	12.7	6.9	0.54
MacConkey	52	36.6	5.8	0.16	40.4	13.5	0.33

NOTE: Six positives were *S. schottmülleri*.

DISCUSSION

E. typhosa may be overgrown by *Esch. coli* when the two organisms are streaked on solid media containing lactose and an indicator for differentiation. Since lactose provides an extra source of energy for *Esch. coli*, these media favor the growth of *Esch. coli* despite careful streaking. Although Leifson's desoxycholate citrate contains lactose and an indicator, *Esch. coli* is restricted through the combined action of the infusion and the citrate radical; certain aerogenes-like strains, if present, may easily obscure *E. typhosa*. Wilson and Blair found that an excess of sodium sulfite and brilliant green restricted the growth of organisms of the colon group on their medium. Upon further incubation (a total of forty-eight hours) many nonpathogenic organisms, however, appear on the plates.

Endo's medium (original formula and the modification of Robinson and Rettger) permits luxuriant growth of *E. typhosa*, but if colon organisms are present, the red color they produce diffuses through the medium so that the

differential colonial characteristics of *E. typhosa* are obscured. This objection is not encountered in the eosin brilliant green or brilliant green agar media, but since the titration of the dyes is tedious, they are not as practical as the eosin methylene blue or the bile media.

Of all the media, Wilson and Blair's bismuth sulfite medium produces the most easily identifiable colonies of *E. typhosa*. The characteristic colony is umbonate. The colony itself is black with metallic luster and is surrounded by a smoky halo. The blackening is due to the formation of sulfides of bismuth and iron. Growth of *E. typhosa* in the presence of a fermentable carbohydrate (dextrose) results in sulfite being reduced to sulfide. The blackness is intensified by brilliant green.

It has been found that by using a medium which inhibits *Esch. coli* to a great extent it is possible to isolate *E. typhosa* when it is mixed with *Esch. coli* in a proportion of 1 to 1,000 (see Table II). It would appear then that the reason why the older media were not so successful in the isolation of *E. typhosa* was because *Esch. coli* is not so selectively repressed as it is by the bismuth sulfite and desoxycholate citrate media.

Where *E. typhosa* is greatly outnumbered by other organisms, isolation may be accomplished by using fluid enrichment media. Leifson's selenite medium closely approaches the ideal for this purpose, since the greatest number of positive typhoid results have been obtained with it (see Table IV).

CONCLUSION

The relative value of any liquid medium is dependent on the selectivity of the solid medium upon which it is subsequently streaked. In this study it has been found that the combined use of Leifson's selenite "F" broth and Wilson and Blair's bismuth sulfite agar provides the most satisfactory combination for the isolation of *E. typhosa* from fecal specimens. Beef bile adjusted to a pH of 6.5 has been found a most satisfactory preservative and enrichment medium for the transportation of specimens to the laboratory.

The use of multiple media in the isolation of *E. typhosa* from stool specimens is costly in materials and time. When an examination is made primarily for typhoid or paratyphoid bacilli, our experience indicates that the use of Wilson and Blair's plating medium and enrichment of specimens in Leifson's selenite broth obviate this necessity and provide a method which all evidence indicates is practically perfect for this purpose. Where the examination is for dysentery bacilli, other and more suitable media for this purpose should be used.

REFERENCES

1. Chesney, A. M.: The Use of Phenol Red and Brom-cresol Purple as Indicators in the Bacteriological Examination of Stools, J. Exper. Med. **35:** 181, 1922.
2. Endo, S.: Ueber ein verfahren zum nachweis der Typhusbazillen, Zentralbl. f. Bakt. **35:** 109, 1904.
3. Holt-Harris, J. E., and Teague, O.: New Culture Medium for the Isolation of *Bacillus typhosus* From Stools, J. Infect. Dis. **18:** 596, 1916. (Difco formula.)*
4. Kauffmann, F.: Ein kombiniertes Anreicherungsverfahren für Typhus- und Paratyphusbazillen, Zentralbl. f. Bakt. Abt. 1, **119:** 148, 1930.

5. Krumwiede, C. J., Pratt, J. S., and McWilliams, Helen I.: The Use of Brilliant Green for the Isolation of Typhoid and Paratyphoid Bacilli From Feces, J. Infect. Dis. **18**: 1, 1916.
6. Leifson, E.: New Culture Media Based on Sodium Desoxycholate for Isolation of Intestinal Pathogens and for the Enumeration of Colon Bacilli in Milk and Water, J. Path. & Bact. **40**: 581, 1935.
7. Leifson, E.: New Selenite Enrichment Media for the Isolation of Typhoid and Paratyphoid (Salmonella) Bacilli, Am. J. Hyg. **24**: 423, 1936.
8. MacConkey, A. T.: Lactose-Fermenting Bacteria in Faeces, J. Hyg. **5**: 333, 1905. (Difco formula.)*
Bile Salt Media and Their Advantages in Some Bacteriological Examinations, J. Hyg. **8**: 322, 1908.
9. Müller, L.: Un nouveau milieu d'enrichissement pour la recherche du bacilli typhique et des paratyphiques, Compt. rend. Soc. de biol. **89**: 434, 1923.
10. Perry, C. A., Hajna, A. A., and Robinson, H.: A Comparative Study of Preservative Media for Delayed Examination of Typhoid and Paratyphoid Bacilli (Unpublished), 1933.
11. Robinson, H. C., and Rettger, L. F.: Studies on the Use of Brilliant Green and a Modified Endo's Medium, in the Isolation of *Bacillus typhosus* from Feces, J. M. Research **34**: 363, 1916.
12. Ruys, A. C.: Ein Brilliantgrün-Medium für die Isolierung von Paratyphus-B-Bakterien aus Stuhl und Urin, Zentralbl. f. Bakt. (Abt. 1) **132**: 349, 1934.
13. Teague, O., and Clurman, A. W.: An Improved Brilliant Green Culture Medium for the Isolation of Typhoid Bacilli From Stools, J. Infect. Dis. **18**: 647, 1916.
14. Wilson, W. J., and Blair, E. M. McV.: Use of a Glucose Bismuth Sulphite Iron Medium for the Isolation of *B. typhosus* and *B. proteus*, J. Hyg. **26**: 374, 1927.
Further Experience of the Bismuth Sulphite Media in the Isolation of *Bacillus typhosus* and *B. paratyphosus B* From Faeces, Sewage and Water, J. Hyg. **31**: 138, 1931.

*Manual of Dehydrated Culture Media and Reagents, Ed. 5, 1935, Difco Laboratories, Detroit, Mich.

AN APPARATUS FOR OPENING KEIDEL TUBES*

ROBERT A. GREENE, PH.D., AND EDWARD L. BREAZEALE, B.S.A., TUCSON, ARIZ.

A LARGE proportion of the blood specimens received by the Arizona State Laboratory are submitted in Keidel tubes. After employing various methods of opening these tubes (with indifferent success) and after several tubes had shattered during the operation, it occurred to us that an apparatus might be constructed which would simplify this operation and reduce the possibility of contamination of the specimen in cases where cultures were to be made from the clot.

The apparatus which was constructed is illustrated in Fig. 1, and the wiring diagram is given in Fig. 2. A "hot wire" is used to remove the upper portion of the Keidel tube, which is held in place by a burette clamp. *A* is an "on-off" switch; *B* is a 600-watt heating element (as used in reflector heaters) mounted in a lamp socket, which serves as a resistance coil; *C* is a piece of 20-gauge nichrome wire, attached to a terminal post at one end and to a wooden handle at the other. A block of wood, 9⅜ by 8⅜ by 1½ inches, serves as a base.

The length and gauge of the nichrome wire will affect the size of the resistance coil to be used. If a resistance of 600 watts is too great, a portion of the

*From the Arizona State Laboratory, University of Arizona, Tucson.
Received for publication, December 7, 1937.

wire on the heating element may be removed; if too small, a larger unit may be employed. An ordinary rheostat of the sliding contact type and of proper size would be very satisfactory, but would be more expensive. The total cost of this apparatus (exclusive of the time required for construction) was $1.50.

To operate, the Keidel tube is placed in the burette clamp, the loop of nichrome wire is slipped around the tube, taking care that the wire in the loop is not in direct contact with another portion of the wire; the loop is tightened by drawing the wooden handle. The current is turned on and contact is made by firmly pressing the wire to a second terminal post. The wire becomes red

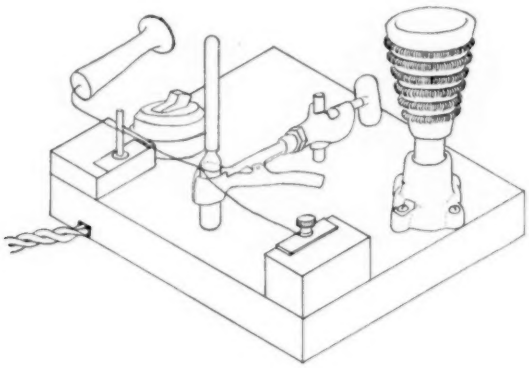


Fig. 1.

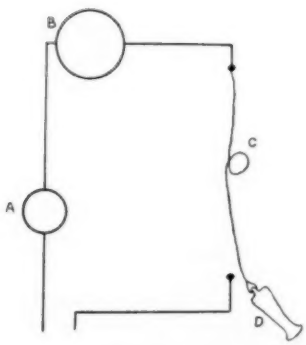


Fig. 2.

hot and after the glass has been heated, a small amount of water is allowed to drop on the glass tube which breaks off smoothly.

If it is desired to make a culture of the clot, the tubes may be scrubbed with mercuric chloride, alcohol, or any suitable germicide, and sterile water may be employed.

This apparatus has given extremely satisfactory results in this laboratory. It has reduced both the time required for opening the tubes as well as the possibility of contamination of the specimen.

We are indebted to John Breazeale who made the drawings; to Jerry Cribbs and Gordon Nielson, who assisted in the construction of the apparatus.

A SIMPLE PROCEDURE FOR THE DIAGNOSTIC CULTURE OF TUBERCLE BACILLI*

H. J. Corper, M.D., Denver, Colo.

THE universal use of the diagnostic culture method for finding small numbers of tubercle bacilli in pathologic specimens has been hampered formerly by the lack of suitable simple devices. These have become available now in slow stages and have been developed laboriously even though the media for culture were proved equal to the guinea pig in efficiency as early as 1929.[1] It is not the purpose of this paper to go over the detailed evidence accumulated during the past decade to prove the obvious, but rather to present available details for preparing the most serviceable reagents and media for diagnostic purposes, and a simple unencumbered procedure which should make the culture method available to all physicians desiring to utilize this important diagnostic process. The method to be described can be used also by supply firms catering to physicians and laboratories not equipped to prepare their own materials or not desiring to do so.

For the sake of simplicity, the subject of cultivating small numbers of tubercle bacilli[2] from contaminated tuberculous materials, such as sputum, urine, pus, etc., may be divided into three phases: (1) the reagent used to destroy contaminating microorganisms; (2) the nutrient media essential to the cultivation of tubercle bacilli when present in small numbers; and (3) the technical details essential to the success of maintaining water and air balance for sufficient periods of time to insure the successful growth of the tubercle bacilli.

1. *The Reagent Used to Destroy Contaminating Organisms.*—After an exhaustive study of a large number of reagents intended to destroy the contaminating organisms found in tuberculous materials, it was found previously that an equal volume of 5 per cent oxalic acid solution brought in intimate contact with the material for thirty minutes to one hour at 37° C. (incubator temperature) proved superior to other reagents, such as 6 per cent sulfuric acid, 3 per cent hydrochloric acid or sodium hydroxide. The oxalic acid reagent also possessed the advantage of being obtainable in pure crystalline form, easily weighed, and stable.[3] This reagent has been used continuously since 1930 in practical tests, and we consider it the most desirable one available for the purpose. However, the past success with the oxalic acid reagent used at incubator temperature led us to hope that it might be useful also at room temperature where speed was not an item and where it could fit in with the day's routine. This accordingly was tested out, and it was found that an equal volume or two

*From the Research Department, National Jewish Hospital, Denver.
Received for publication, December 27, 1937.

volumes of 5 per cent oxalic acid solution added to sputum would not interfere with the recovery of small numbers of tubercle bacilli when the reagent was intimately mixed with the sputum and kept at room temperature (about 25° C.) up to five hours, and that contaminations were few after one hour's exposure to the oxalic acid. An exposure beyond five hours, however, proved detrimental to the tubercle bacilli. An illustrative experiment with 5 tuberculous sputums, using 3 tubes of egg yolk medium for each interval planting, showed for sputum No. 1, 1 tube of 3 contaminated after one hour's exposure, 1 tube of 3 after three hours' exposure, 1 tube after four hours' exposure, and 1 after five hours' exposure; while with sputum No. 2, only 1 tube contaminated after three hours' exposure; with sputum No. 3, one tube contaminated at the one-hour exposure, 1 at two hours, and 1 at five hours; sputum Nos. 4 and 5 only showed 1 contamination after one and two hours' exposure, respectively. These and other similar experiments led to the conclusion that an exposure to one or two volumes of 5 per cent oxalic acid solution for about two hours at room temperature with intimate mixing of the specimen and oxalic acid could be recommended for practical purposes, although contact up to four hours is safe and might be advisable for heavily contaminated materials.

Since the most satisfactory method of stopping the action of the oxalic acid at the desired time was to neutralize the acid, as shown in earlier experiments, and since the use of a strong alkali, such as sodium hydroxide, might both serve to produce an undesirable hypertonicity and to change the reaction to the alkaline side, which might prove detrimental to the growth of the bacilli, a well-buffered mixture was sought that would not prove detrimental to the tubercle bacilli and still serve to neutralize the oxalic acid sufficiently to insure a cessation of its action. An excellent buffered solution for this purpose was found by using the basic essentials of a nonprotein medium used for growing heavily planted tubercle bacilli for experimental investigations and the preparation of purified tuberculins. Such a medium was described by Wong and Weinzirl[4] in 1936, and we omitted the malic acid, ammonia, glycerol, and glucose from this medium for our purposes. The composition of this buffered solution as finally used for neutralizing and diluting the oxalic acid is as follows:

BUFFERED NEUTRALIZING AND DILUTING SOLUTION

Ammonium citrate	5.00 gm.
Monopotassium acid phosphate	6.00 gm.
Sodium carbonate (anhydrous)	2.00 gm.
Sodium chloride	2.00 gm.
Magnesium sulfate	1.00 gm.
Ferric ammonium citrate	0.05 gm.
Distilled water to make	1000.00 c.c.

The solution is sterilized by autoclaving and has an approximate pH of about 7.0 when completed. It is capable of readily neutralizing the oxalic acid solution without swinging over to an alkaline reaction if used for washing purposes, as will be detailed later in describing its use in the technical phase below.

2. *The Nutrient Media for Growing Small Numbers of Tubercle Bacilli.*—In the past, many suitable media for promoting the growth of small numbers of tubercle bacilli have been described, but the efficiency of many of these had not been accurately evaluated quantitatively by those suggesting their use. Many of them were of complicated composition, containing a large number of expensive and unessential components.[5, 6]

In our own studies,[7] we aimed at two important points which were studied quantitatively: to find a nutrient with all the essentials to support growth from very small plantings of tubercle bacilli, and to find the simplest form in which it could be used for this purpose. As a result of these studies, we at first described a simple glycerol water crystal violet potato cylinder medium for diagnostic culture purposes.[8] It consisted essentially of a clean potato cylinder cut to suitable size, neutralized, and slightly colored by soaking for about one to two hours in a freshly mixed 0.0015 per cent standard crystal violet (to exert a slight static action on contaminants not destroyed by the acid treatment) in 1 per cent sodium carbonate solution (prepared from the pure anhydrous salt). Immediately after cutting, the potato cylinder is placed in water to avoid discoloration and deterioration from air contact. The prepared cylinder is placed in 1.5 c.c. of 6 per cent aqueous solution of pure glycerol in water and is sterilized in an autoclave at 15 pounds pressure for thirty minutes (excessive or prolonged heating being avoided).

As a result of further studies, a second good nutrient medium was described in the inspissated glycerol egg yolk medium[9] prepared from fresh egg yolks (separated from the white which was found to be a poor nutrient for supporting the growth of small numbers of tubercle bacilli), about 25 per cent water (neutral), and sufficient pure glycerol (4 gm. to 100 gm. of yolk and 33 c.c. of water). If a colored medium is desired to make the routine examination for colonies easier, an inert dye or pigment may be added; 3 c.c. of 1 per cent Congo red in water to 100 c.c. of the egg mixture (making 0.03 per cent) was found suitable for this purpose. The tubed mixture is sterilized in a slanted position by inspissating for one hour on three successive days at 85° C. It also can be sterilized by a single autoclaving if provision is made to avoid the presence of bubbles in the completed medium.

In comparative practical tests with these two media, the potato cylinder and the inspissated egg yolk, using 100 microscopic negative sputums tested by two groups of workers, approximately the same results were obtained and both media proved to be good nutrients about equal in value.[10] There was, however, this slight difference noted in that the positive findings on the inspissated egg yolk medium could be noted in many cases about a week earlier than on the potato cylinder medium, although the final positive results were about the same. On the other hand, some workers preferred the potato for the ease with which colonies could be seen. For this and other like unimportant reasons, the choice between the two becomes a matter for each individual to decide.

3. *The Essential Technical Conditions Required for Obtaining Growth from Small Numbers of Tubercle Bacilli.*—In addition to the use of a good nutrient

approximately neutral in reaction (about pH 7.0), conditions close to isotonicity, and a readily accessible supply of air maintained throughout the entire period up to about two to three months required for the successful growth from small plants of tubercle bacilli at 37° C., provision had to be made to prevent evaporation of the liquid from the medium and to avoid contamination of the medium from outside sources. In a previous report,[11] a sterile cork was advised as being satisfactory and simplifying the required technique. It was pointed out at that time that the problem of maintaining water and air balance in culture tubes and culture receptacles appeared to be one of preventing the conduction of moisture from these receptacles by avoiding the use of moisture absorbent materials, or by satisfactorily impregnating these with a completely nonabsorbent and water impervious material, such as paraffin. This can be attained without appreciable influence upon adequate gaseous exchange sufficient to maintain conditions required for the multiplication of slow growing microorganisms, such as the tubercle bacillus. With the recent advent of better glass receptacles on the market, it appeared worth while for economic reasons as well as simplicity to try to replace the cotton plugged culture tube by the more simply handled and less expensive screw-capped culture vial, and, if possible, to eliminate the use of the paraffined cotton plug. A series of tests to determine air exchange by using a suitable manometric gauge showed that many of the liners in the screw caps did not provide adequate air passage and that it would be advisable to replace them by a standard cut compressed cork. It was also determined that even a small amount of paraffin or oil might make these corks impervious to air, and that a water moist cork could be impervious, whereas a dry cork proved pervious. It was also found that a moist cork disk in the screw cap would dry out readily in the incubator and again become pervious, even though the vial or bottle contained large volumes of liquid. At the same time, the cork liner in the metal screw cap would insure against evaporation of the liquid contained in the bottle for months at incubator temperature and, if the screw cap was not screwed down too tightly, would insure adequate air exchange and exclude outside contamination for months of incubation (experiments extending up to twelve months with broth cultures have proved the satisfaction of this method). The simplicity over the use of flasks and culture tubes closed by the usual methods is quite striking. For the diagnostic culture method, vials were bought by the gross and chosen for their ability to withstand both dry sterilizing and moist autoclaving without cracking or breaking. Such vials are readily obtained on the market for about half the price of bacteriologic culture tubes. Each new batch should be tested for heat resistance before use.

PREPARATION OF THE BOTTLES

Two sizes of screw cap bottles were used in conducting the tests, although the sizes may be varied to suit the individual choice of the worker. The bottles we used (see Figs. 1 and 2) are commonly known in trade as screw cap "homeopathic vials." The small pad in the metal screw cap was replaced by a clean compressed cork pad made by cutting small round disks of suitable size to fit snugly in the cap. A round steel punch, a wooden mallet, and wooden block

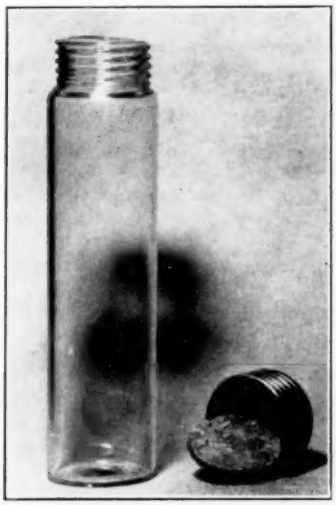


Fig. 1.—Illustrates one of the large bottles (vials) of about 35 c.c. content, showing the metal screw cap removed and the 1/16 inch compressed cork disk (cut by means of a steel punch) which fits snugly in the cap.

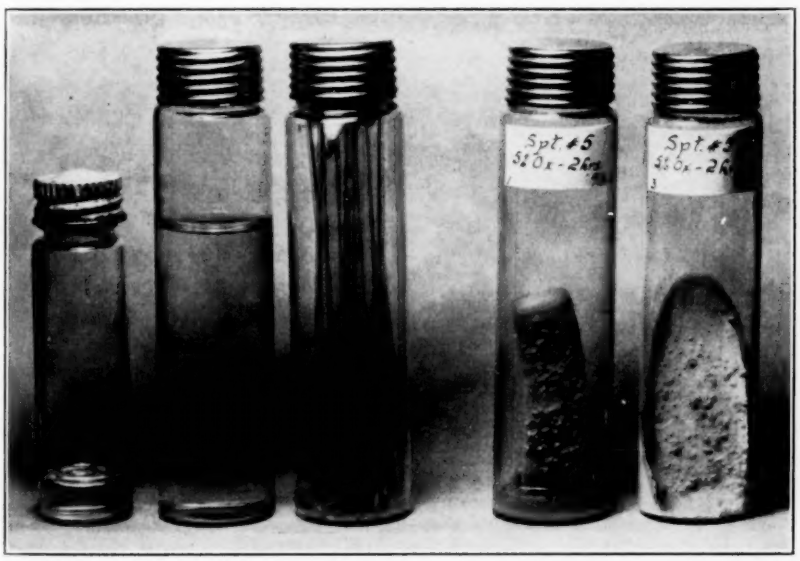


Fig. 2.—Shows the essential equipment for performing the room temperature oxalic acid treatment for isolating small numbers of tubercle bacilli. The small bottle on the left contains about 1.5 to 2 c.c. of sterile 5 per cent oxalic acid solution for destroying contaminators in the specimen. The second larger bottle contains about 25 to 30 c.c. of the neutral buffered solution designed to stop the action of the oxalic acid after two hours' treatment at room temperature. The third bottle from the left contains four sterile cotton plugged pipettes for the manipulation of the sputum, buffer solution, and sediment. The fourth and fifth bottles show the growth of the tubercle bacilli on one of the potato and egg yolk media, respectively, resulting from the cultivation of sputum 5 by the simple procedure outlined. This method replaces the paraffined cotton stopper and the usual bacteriologic test tube by means of the easily handled screw cap, cork lined, bottle (vial).

comprised the apparatus for cutting the disks. The cork was compressed cork sheet (made by the Victor Manufacturing and Gasket Co. of Chicago) obtained in large sheets from a foot to several feet in size. The 1/16 inch thick grade (Victor 32500) was chosen and found free from undesirable fatty, waxy, or oily materials which might interfere with air permeability.

The bottles selected were a small size digestion vial holding about 15 c.c. (2 cm. in diameter by 7 cm. in height) and a large size culture bottle of 35 c.c. capacity (2.5 cm. in diameter by 10 cm. in height).

To obtain complete and thorough initial sterilization of the bottles and cork disks, a few drops of pure ethyl (grain) alcohol were placed in the bottle and on the cork before submitting them to the usual dry sterilization in an oven. Past experience has proved this a wise procedure, especially for obtaining sterility of new glassware, cork material, or heavily contaminated equipment, when the hay bacillus and spore formers are likely to be present. Dry sterilization of the vials is carried on also long enough to insure complete evaporation of all traces of alcohol from the inside of the bottles and cork, but not to the point where the cork is burnt or even slightly charred.

TRIALS WITH THIS METHOD

In order to test the method in practice, 10 sputums from tuberculous patients were collected. A 1 c.c. sample was placed in the digestion bottle containing 1.5 c.c. of 5 per cent oxalic acid solution, using a sterile glass tube for making the transfer and for intimate mixing. The mixture was allowed to stand at room temperature, protected from light, with occasional shaking for two hours. After this, the small (digestion) bottle was filled about two-thirds or three-fourths full by adding the sterile neutral buffer solution described above (and previously sterilized by autoclaving). Then it was allowed to stand for two to three hours, during which time the sediment settled down and the supernatant clear fluid could be withdrawn by another sterile glass tube (one of the 4 originally supplied in one of the large vials). To the sediment was then added some more of the sterile neutral buffer solution up to the two-thirds or three-fourths mark. After thoroughly mixing, the sediment was again allowed to settle, and the clear supernatant fluid again withdrawn by means of the third sterile tube, leaving the sediment to be planted on several (preferably 3 to 4) of the potato or egg yolk medium culture bottles by means of the last (fourth) sterile pipette. In emergency, if pipettes are not supplied with the outfit, clean pipettes can be sterilized by heating them to a high heat but below the melting point of the glass in the Bunsen burner, but every precaution must be taken to permit the tubes to cool well so that the sputum sediment transferred is not heated (60° C. may destroy the bacilli under suitable conditions). The sediment is planted directly on the surface of the potato and egg yolk media since the bacilli must derive their nutrients for growing from this source. To insure this, the bottles may be planted while lying on a slant and be maintained thus for several hours in the incubator or at room temperature to aid their adhering

to the slant surface of the medium. After planting, the screw caps are replaced on the culture vials, taking care not to screw them too tightly. Gentle turning suffices to seal against bacterial contamination and permits ready air access without easy loss of the necessary moisture contained in the media.

Table I shows the results of culturing 10 tuberculous sputums, 5 each by two separate operators, using the simple room temperature digestion technique. The action of the 5 per cent oxalic acid was stopped after two hours with the neutral buffer solution described, and the sediment planted on the potato and egg yolk media. The two photographs included show all the essential equipment required for performing the simple test with the exception of a rubber nipple used on the glass tube pipettes and an incubator to maintain the culture tubes at 37.5° C. (optimum temperature for growing human, bovine, and avian tubercle bacilli).

TABLE I

THE RESULTS OF ISOLATING TUBERCLE BACILLI FROM 10 TUBERCULOUS SPUTUMS BY MEANS OF A SIMPLE PROCEDURE UTILIZING SCREW CAP BOTTLES

SPECIMEN OPERATOR NO. 1	MEDIUM	RESULTS	SPECIMEN OPERATOR NO. 2	MEDIUM	RESULTS
1	Egg Yolk	2^{2*}	6	Egg Yolk	2^{1}
	Potato	3^{3}		Potato	3^{3}
2	Egg Yolk	2^{3}	7	Egg Yolk	2^{3}
	Potato	3^{2}		Potato	3^{3}
3	Egg Yolk	2^{1}	8	Egg Yolk	2^{3}
	Potato	3^{3}		Potato	3^{3}
4	Egg Yolk	2^{1}	9	Egg Yolk	2^{3}
	Potato	5^{2}		Potato	3^{3}
5	Egg Yolk	2^{3}	10	Egg Yolk	2^{3}
	Potato	3^{3}		Potato	3^{2}

*The first numeral given indicates the number of weeks in which the earliest growth was noted with the naked eye, while the exponent indicates the number of vials of a total of 3 of each medium revealing a positive growth. Usually the negative tubes were prevented from growing by contamination.

The findings illustrate the usual run of results with the two media, potato and egg, and with two operators using different equipment and laboratories. The two series of tuberculous sputums were obtained from sanatorium patients.

Attention should be called to the fact that when the presence of non-glycerophilic bovine tubercle bacilli is suspected, the media should include vials of potato or egg yolk medium prepared without glycerol. As has been noted previously, visible growth of the tubercle bacilli can hardly be expected in less than two weeks, even from heavily-laden materials, and the time required increases up to about two months when sparsely laden microscopically negative materials are used. For all practical purposes, culture bottles are not kept after three months, since growth of the bacilli rarely if ever occurs after this time.

It is hoped that this simple method, encompassing the requirements of most institutions and physicians for careful study of suspected tuberculous cases, will result in a more universal adoption of the diagnostic culture method for determining the presence of tubercle bacilli in pathologic specimens.

I wish to thank Mr. Ray Stoner and Mr. David Miller for assisting with the technical phases of this study.

REFERENCES

1. Corper, H. J., and Uyei, Nao: Further Observations With a New Method for Cultivating Tubercle Bacilli: A Comparison With Guinea Pig Inoculation and Petroff's Method, J. Lab. & Clin. Med. **14**: 393, 1929.
2. Corper, H. J.: The Cultivation of Tubercle Bacilli, Am. J. M. Technol. **2**: 157, 1936.
3. Corper, H. J., and Uyei, Nao: Oxalic Acid as a Reagent for Isolating Tubercle Bacilli and a Study of the Growth of Acid Fast Non-Pathogens on Different Mediums With Their Reactions to Chemical Reagents, J. Lab. & Clin. Med. **15**: 348, 1930.
4. Wong, Sam, and Weinzirl, John: An Inexpensive Synthetic Medium for Growing Mycobacterium Tuberculosis, Am. Rev. Tubere. **33**: 577, 1936.
5. Cohn, Maurice L.: An Analysis of Loewenstein's Method for Isolating Tubercle Bacilli From Blood, J. Infect. Dis. **52**: 214, 1933.
6. Corper, H. J., and Damerow, A. P.: The Question of Tubercle Bacilli in the Blood in Advanced Pulmonary Tuberculosis, Am. Rev. Tubere. **28**: 118, 1933.
7. Uyei, Nao: The Nature of the Growth-Promoting Active Principle in the Potato in the Cultivation of Bacteria and Especially of the Tubercle Bacillus, Am. Rev. Tubere. **22**: 203, 1930.
8. Corper, H. J., and Uyei, Nao: A Simple Glycerol Water Crystal Violet Potato Cylinder Medium for Diagnostic Cultures of Tubercle Bacilli, Arch. Path. **7**: 835, 1929.
9. Corper, H. J., and Cohn, Maurice L.: The Nutrient Quality of Eggs for Growing Tubercle Bacilli, Am. J. Hyg. **18**: 1, 1933.
10. Corper, H. J., and Cohn, Maurice L.: A Routine Clinical Examination for Tubercle Bacilli in Microscopic Negative Sputums by Various Culture Methods, J. Lab. & Clin. Med. **18**: 515, 1933.
11. Corper, H. J., and Cohn, Maurice L.: Maintaining Water and Air Balance During Prolonged Incubation, J. Lab. & Clin. Med. **19**: 899, 1934.

A PORTABLE AIR-USING METABOLIMETER*

C. V. Perrill, M.S., and H. C. Sanderson, A.B., Chicago, Ill.

THE possibility of using air in closed-circuit apparatus for determining the metabolism of human beings has been considered in a previous paper.[1] The authors found, in a series of 60 tests, that air could be substituted for oxygen without diminishing the accuracy of the test. It was also pointed out that such apparatus might be of considerable value in remote regions where oxygen is difficult to obtain. Such an apparatus is also of interest to the American practitioner, for the results obtained with it under certain conditions may be superior to those obtained with the conventional oxygen-using apparatus.

The senior author has been particularly interested in designing apparatus for use in India, a "remote region" in which he has spent a number of years, and the accompanying figures illustrate an apparatus which has been shipped to India. The machine differs essentially from the conventional oxygen-using apparatus by the arrangement of a tank, 10 by 10 by 18 inches, around a spirometer water jacket which allows enough air to be enclosed within the system to permit the subject to breathe comfortably for six minutes or longer. Ade-

*From the Department of Physiological Chemistry, Northwestern University Medical School, Chicago.

Received for publication, December 20, 1937.

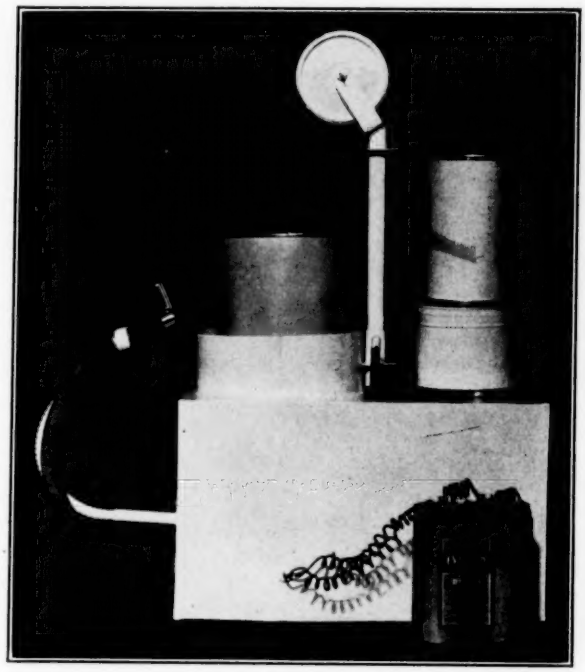


Fig. 1.

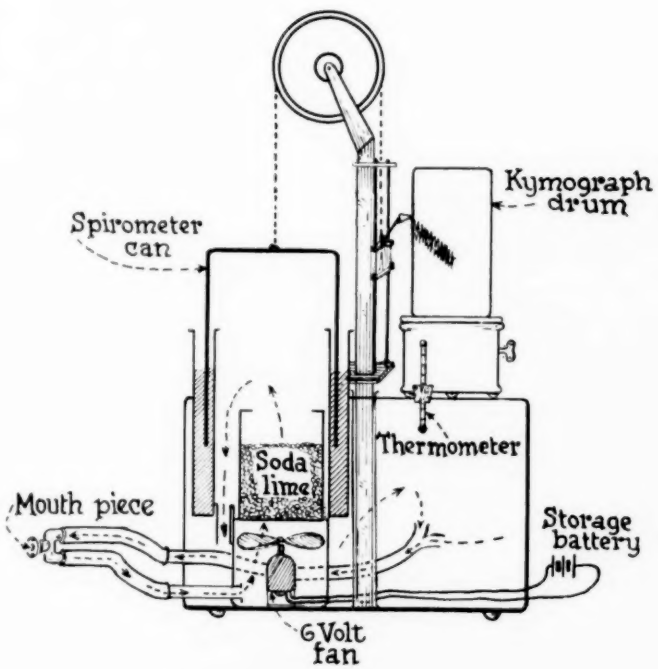

Kymograph drum
Spirometer can
Thermometer
Soda lime
Mouth piece
Storage battery
6 Volt fan

Fig. 2.

quate air circulation within the apparatus is insured by the use of a small 6-volt motor fan, operated by the dry cells shown in Fig. 1, or by an automobile battery. The oxygen consumption is measured by the fall of the spirometer which is recorded on a kymograph drum in the usual manner.

The experimenters attempted to make this apparatus as sturdy and as compact as possible. Compactness was obtained in several ways: First, the total volume of the system is reduced to 30 liters, as observations on other machines have led the writers to believe that 30 liters is, under ordinary circumstances, an ample volume of air for an average six-minute test. Second, the spirometer and water jacket are lowered into the surrounding tank, until the base rests upon the floor of this tank. This not only places most of the spirometer water jacket out of sight, but also proportionately reduces the height of the upright supporting the pulley wheel. Further compactness is obtained by telescoping this upright support into a tube of slightly larger size, which extends down to the floor of the surrounding tank. During shipment of the machine the upright support with the pulley wheel is removed and placed along the side of the tank. The tube which received the upright is soldered firmly against the water jacket, as well as to the roof and floor of the tank. The ends of the tank are closed with heavy galvanized sheet steel. Two copper breathing tubes are soldered directly to the surrounding tank and motor-housing, as well as to their points of exit. At the base of the water jacket a small six-volt motor is housed, mounted in three layers of live rubber, which dampen vibrations effectively.

Since this machine was designed to resist the Indian climate, the inside of the tank is covered with two coats of enamel, and the outside with four coats. In addition, the soda-lime can received a coat of acid-resisting paint, and the spirometer bell and moving parts are both copper plated and chrome plated.

The kymograph was constructed by housing a clockwork in a suitable dust-proof can. This mechanism rotates a turntable at a speed suitable for the use of a tall, but light, drum which accommodates a paper 6½ by 13 inches in size. The machine and all accessories were packed for shipment to India in a small, light trunk.

This machine was carefully checked mechanically before shipment. The spirometer was calibrated accurately by measuring the distance traversed by the pen on the kymograph drum when air was added or withdrawn in 500 ml. increments from the machine. Tests with a subject gave results which are similar to those of our other oxygen and air-using machines. For example:

Comparison of Six-Minute Tests

SUBJECT	MINUTE-VOLUME		DIFFERENCE	
	AIR	OXYGEN	ML.	PER CENT
X	315 ml.	310 ml.	5	1.6
X	323	304	19	6.3
X	277	288	−11	−3.8
Totals	915 ml.	902 ml.		
Average Difference			4 ml.	1.4 per cent

Temperature changes within this machine were found to be small enough so that they could be entirely disregarded after a preliminary test had estab-

lished a temperature equilibrium within the machine. An accurate thermometer was, however, provided.

Information received from India indicates that the machine arrived in good condition, and that it should find a field of usefulness in an area where the cost of recharging a small oxygen tank is almost $10.00.

The design of this machine is simple enough to permit the construction of duplicates in India, for the Indian tinsmith is often skilled in working with copper tubing and sheet metals. The materials in this apparatus cost $17.00 (in Chicago). This includes both the clockwork and the six-volt motor, but does not include the cost of labor.

In our previous paper[1] it has been shown that any risk to the subject, due to anoxemia, can be entirely avoided by preventing the oxygen level in the system from dropping below 16 per cent. This may always be insured by constructing the spirometer bell so that its capacity is less than 6 per cent of the total system; thus the bell always reaches the bottom of the water jacket when the period of safe operation draws to a close, provided that the machine is filled with fresh air at the beginning of each test.

Recently the question has been raised concerning the origin of closed-circuit air-using metabolism apparatus used on human beings. We believe that priority should be given to Y. Henderson's apparatus, a description of which appeared in the J. Physiol., Proceedings of the Society xxiv, May 1915.

REFERENCE

1. Perrill, C. V., and Jones, K. K.: Use of Air in Basal Metabolism, Proc. Soc. Exper. Biol. & Med. **36**: 444, 1937.

303 E. Chicago Avenue

A METHOD OF COLLECTING SMALL BLOOD SPECIMENS*

With Special Reference to Microflocculation Tests for Syphilis

John A. V. Davies, M.D., Boston, Mass.

THE number of methods for making tests on small specimens of blood[1, 2] is increasing steadily. They are of considerable practical importance, especially in pediatrics. When blood for this purpose can be obtained by skin puncture, the patient usually objects less to the procedure, particularly if the veins are not easily accessible to venipuncture. Blood specimens obtained by skin puncture have been collected in a variety of receptacles: small test tubes; long Wright tubes; test tubes drawn out to a capillary tip, with a hole in the side for an air vent; small glass tubing plugged with plasticine; glass tubing sealed with rubber bands or with rubber policemen;[3] and large capillary tubes sealed in a flame or with sealing wax. The purpose of this communication is to describe the receptacle used at the Infants' and Children's Hospitals in Boston for the collection of blood specimens for microflocculation tests for syphilis.[4-6] Because of its convenience it may find a wider field of usefulness.†

The receptacle consists of a clean, dry, strong-walled glass tube, measuring 8 cm. in length by 2.5 to 2.75 mm. in inside diameter. The outside diameter is about 4 mm. and the total capacity a little over 0.3 c.c. Over each end fits a clean, flexible rubber cap such as is commonly used for corking vaccine and serum bottles (see Fig. 1). It has a hole 6 mm. deep and 3 mm. wide, except at the outermost portion, where it is somewhat wider. The stem of the cap is 6 mm. in diameter. The closed end supports a circular flange, giving it a total diameter of 9 or 10 mm. This flange assists in pressing the cap on the tube and in prying it off. It also protects the tube if accidentally dropped. The filled and capped tube is of such a length that it fits inside an ordinary "Wassermann" test tube and can be safely transported in the routine way to the laboratory, without special precautions.

The tubes should be filled two-thirds to three-fourths full by the attending physician or nurse. Usually no difficulty is experienced in obtaining this amount of blood from the finger of an adult or child, but in the case of an infant the following suggestions should be borne in mind:

*From the Infants' and Children's Hospitals, Boston, the Department of Pediatrics, Harvard Medical School, and the Wassermann Laboratory, Harvard Medical School.

Received for publication, December 28, 1937.

†E.g., other microtests for syphilis, or the quantitative determination in small amounts of serum or plasma of the protein, phosphorus, chloride, or ascorbic acid[7] content.

1. Remove both caps from the tube.

2. The skin of the great toe selected should be warm and pink. If necessary, wrap a warm towel around the foot or massage it briskly. Holding the foot in a dependent position helps.

3. Moisten the skin of the great toe with 70 per cent alcohol.

4. With a sharp V-shaped blade (e.g., a Bard-Parker No. 11,* kept in Bard-Parker sterilizing solution or formaldehyde germicide), puncture the ball of the great toe fairly deeply in one or two places so that blood can be expressed without much squeezing.

5. Wipe off the alcohol and the first drop of blood. Do not restrain the leg so as to impede the flow of blood. After puncturing the skin, wait a few seconds before starting to "milk" the toe. Hold the collection tube horizontally or at a slight inclination while filling it. Try to maintain an unbroken column of blood in the tube.

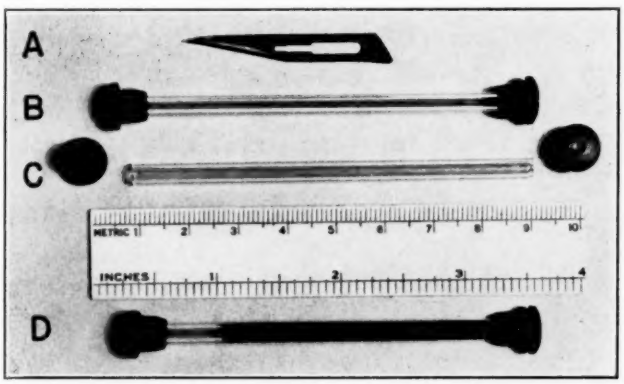


Fig. 1.—*A*, blade recommended for skin puncture; *B*, empty capped tube; *C*, rubber cap; *D*, capped collection tube containing blood.

6. After filling the tube and replacing the rubber caps, apply pressure over the puncture and elevate the foot until oozing ceases.

The number of tubes to be filled will depend largely on the microtest for which the blood is collected. The "Davies-Hinton" microflocculation tests require either 0.2 c.c. or approximately 0.05 c.c. of serum. If the "micro" test is to be performed, the necessary 0.2 c.c. of serum can usually be obtained from two or three collection tubes three-fourths full. However, since the smaller amount of serum (0.05 c.c.) required for the "capillary" test gives results which appear to be fully as accurate, this latter method is usually the one of choice and, except in the case of some premature newborn, or dehydrated infants with high hematocrit readings or poor clot retraction, one of the collection tubes almost always supplies ample serum.

In the laboratory, the technician makes certain that the rubber caps fit snugly on the tubes before centrifuging the specimens at high speed for five

*This blade is less painful and more efficient than many instruments in common use.

minutes in labelled test tubes. If, at the end of this time, the clot is found to be still adherent to the walls of the tube, inverting and recentrifuging the specimen toward the opposite end of the collection tube will, with few exceptions, result in satisfactory separation of the serum from the clot. Should shreds of the clot still cling to the walls of the tube, it may be necessary to loosen them with a toothpick or wire before final centrifugation. The cap at the serum end of the tube is now removed and the tube is scored just above the level of the clot with a sharp, thin file such as is often supplied for opening glass ampoules, or with an ordinary small, sharp, triangular file. The tube is carefully broken so as to retain the serum. The correct amount of serum (0.05 c.c.) is then run into a capillary tube for the "capillary" test, or 0.2 c.c. is drawn up into a graduated capillary pipette for the "micro" test.

Should one wish to do so, the entire specimen, after centrifugation, may be inactivated at 56° C. for thirty minutes before the supernatant serum is withdrawn. Unless agitated or allowed to stand a long time, the serum is not harmed by contact with the clot in the bottom of the tube and one step in the procedure is thereby eliminated.

Since the "Davies-Hinton" microflocculation tests rely on a mixture of the correct proportions of inactivated serum and a stable glycerinated antigen, the exact quantity of serum (within the lower limit of 0.05 c.c.) need not be measured, provided the correct proportions of serum and glycerinated antigen or "indicator" are maintained and a convenient column of the mixture results. One can, therefore, perform the entire test in these tubes, if care is taken to see that the rubber caps fit tightly. Moreover, complement fixation tests, employing small amounts of serum, have been satisfactorily carried out in these tubes.

Serum or plasma for more exact quantitative analyses can be aspirated from these collection tubes by means of a long, slender needle attached to a micropipette which is clamped in a vertical position. The opposite end of this pipette is connected to an oiled syringe by means of short rubber tubing and glass tubing bent like an inverted hook so that the syringe lies within easy reach of the technician.

In addition to their convenience, with these collection tubes there appears to be less likelihood of hemolysis of blood specimens during transportation than with the regular "Wassermann" test tubes. The result is fewer "unsatisfactory" reports due to hemolysis. Specimens mailed in Florida have arrived in Boston in excellent condition.

In estimating the value of a receptacle for the collection of blood specimens for microtests for syphilis, the following criteria should be considered: convenience to the clinician; freedom from hemolysis, contamination, evaporation, breakage, and leakage; maximum yield of serum; facility of transportation through the mails; economy; and ease of handling in the laboratory. In our experience, this collection tube has fulfilled these requirements.

REFERENCES

1. Drucker, Paul, and Cullen, Glenn E.: A Simple Method for Obtaining Cutaneous (Capillary) Blood From Infants and Adults for Colorimetric pH Determination, J. Biol. Chem. **64**: 221, 1925.
2. Abrahamson, E. M.: A Convenient Method of Securing Blood for Analysis, Science **86**: 202, 1937.
3. Rein, C. R., and Le Moine, M.: Value of Kline Precipitation Test for Detection of Syphilis in Applicants for Life Insurance, Urol. & Cutan. Rev. **39**: 233, 1935.
4. Editorial: The Efficiency of Serodiagnostic Tests for Syphilis, J. A. M. A. **108**: 728, 1937.
5. Davies, John A. V.: A Microflocculation Test for Syphilis, J. Lab. & Clin. Med. **22**: 959, 1937.
6. Davies, John A. V.: A "Rapid" Micro-Hinton and Capillary Hinton Test for Syphilis, J. Pediat. **10**: 802, 1937.
7. Mindlin, R. L., and Butler, A. M.: The Determination of Ascorbic Acid in Plasma: A Macro and Micro Method, J. Biol. Chem. **122**: 673, 1938.

101 Bay State Road

A SIMPLE METHOD FOR THE QUALITATIVE DETERMINATION OF URINARY PROTEIN*

Joseph E. Smadel, M.D., New York, N. Y.
With the Technical Assistance of Gilbert de Mello

A SIMPLE rapid method for the qualitative determination of protein in small volumes of urine has been evolved during studies on experimental nephritis in rats. This technique is applicable to human urine and can be used to advantage when many specimens are to be tested daily. The procedure makes use of the well-established protein precipitating ability of trichloracetic acid;[1] it is carried out in a single step at room temperature and gives immediate results which agree closely with quantitative protein determinations by the method of Shevky and Stafford.[2]

A 10 per cent aqueous solution of trichloracetic acid is added in quantity equal to the specimen to be tested. The volume of urine may be as small as 0.1 c.c., but amounts of 0.5 c.c. or 1.0 c.c. are preferable. Glass test tubes having an inside diameter of 4 to 10 mm. are used, depending on the volumes employed. Usually 4 mm. tubes are placed in a rack, 0.5 c.c. of each sample is pipetted into a tube, after which 0.5 c.c. of the reagent is added by means of a graduated pipette or burette. The two solutions may be layered and the reaction read as

*From the Hospital of the Rockefeller Institute for Medical Research, New York.
Received for publication, December 31, 1937.

a ring test, or they may be mixed and the amount of flocculation estimated. The degree of precipitation varies from a faint turbidity to a solid coagulum; the usual designations of trace to ++++ are convenient. Fig. 1 illustrates the reactions in urines with different amounts of protein.

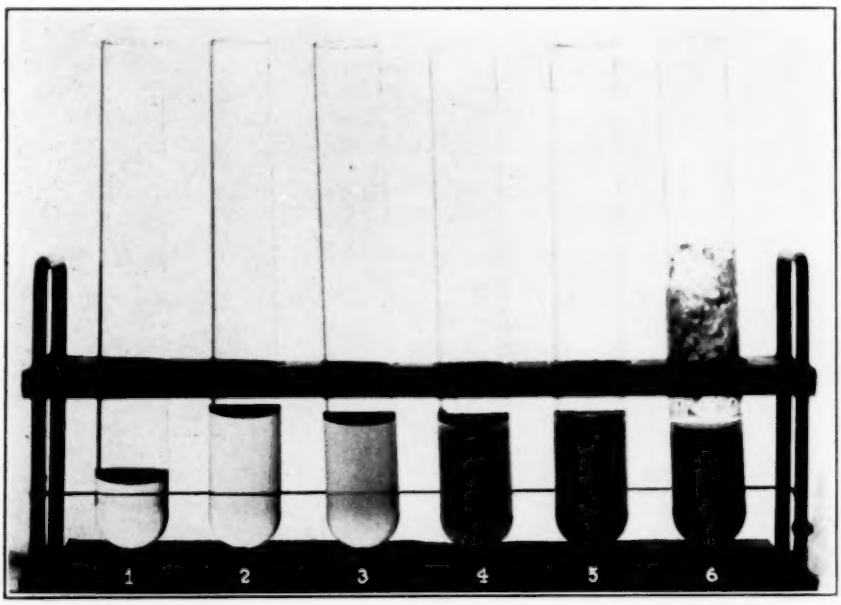


Fig. 1.—Precipitation of urinary protein by 10 per cent trichloracetic acid. Tubes 1 and 2 contain normal urine; the first is untreated, the second contains an equal volume of reagent. Tubes 3 to 6 illustrate increasing strength of reactions from + to ++++, respectively. Tube 6 shows coagulated protein deposited on its wall after being shaken.

In Table I are presented the comparative results obtained with 12 urine specimens as determined by the present method and other qualitative methods in common use,[3] together with the quantitative values.

TABLE I

DETERMINATION OF URINARY PROTEIN BY VARIOUS METHODS

URINE SPECIMEN	SHEVKY-STAFFORD PER CENT	10 PER CENT TRICHLOR-ACETIC	HEAT AND ACETIC	HNO_3 (HELLER'S RING TEST)	ROBERTS' TEST	PICRIC ACID
Human No. 1	Negative	Negative	Negative	Negative	Negative	Negative
Human No. 2	0.04	T	F.T.	H.T.	T	T
Human No. 3	0.17	H.T.	V.F.T.	++	+	H.T.
Human No. 4	0.18	H.T.	Negative	T	T	H.T.
Human No. 5	0.51	+	V.F.T.	++	+	+
Human No. 6	1.91	+++	+++	++++	+++	+++
Rat No. 7	0.14	T	H.T.	+	H.T.	T
Rat No. 8	0.34	H.T.	T	T	H.T.	T
Rat No. 9	0.40	+	H.T.	++	+	+
Rat No. 10	0.69	+	+	+++	++	+++
Rat No. 11	0.92	++	+	+++	++	+++
Rat No. 12	1.95	+++	++	+++	++	++

V.F.T. = Very faint trace; F.T. = Faint trace; T = Trace; H.T. = Heavy trace.

SUMMARY

Ten per cent trichloracetic acid mixed with equal amounts of urine serves as a convenient qualitative test for proteinuria. The results are less variable than those obtained with most other qualitative methods; the test is more reliable with small volumes of urine. Because this simple procedure can be quickly applied, it saves much time when many specimens must be examined.

REFERENCES

1. (a) Quick, A.: The Estimation of Albumin in Urine. J. Lab. & Clin. Med. 8: 615, 1923. (b) Hiller, A.: Determination of Albumin and Globulin in Urine, Proc. Soc. Exper. Biol. & Med. 24: 385, 1927.
2. Shevky, M. C., and Stafford, D. D.: Clinical Method for Estimation of Protein in Urine and Other Body Fluids, Arch. Int. Med. 32: 222, 1923.
3. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, ed. 8, Philadelphia, 1935, W. B. Saunders Co.

THE USE OF SWABS IMPREGNATED WITH ASCITIC FLUID IN THE LABORATORY DIAGNOSIS OF GONORRHEA*

Robert A. Greene, Ph.D., and Edward L. Breazeale, B.S.A., Tucson, Ariz.

THE presence of gram-negative diplococci in a smear from a suspected gonococcal infection does not necessarily prove that the infection may be due to the gonococcus, particularly, if the specimen is from a female or from a chronic case of long standing. Laboratory confirmation of the physician's diagnosis is frequently complicated by the inability to find typical organisms in a stained preparation, as well as the fact that organisms other than the gonococcus, which are similar in morphology and staining reactions, may be present.

Consequently, increasing emphasis is being placed upon the use of cultural methods for the definite identification of the gonococcus. No particular difficulties are involved if the cultures may be taken at a laboratory; if, however, the material is to be transported for any distance, the chances of isolating the gonococcus are greatly decreased.

The success of the use of serum impregnated swabs in the laboratory diagnosis of diphtheria (Folger-Solé,[1] Brahdy[2] technique) suggested that a similar modification of the method of Park and Williams[3] might prove a valuable aid in the diagnosis of gonorrhea.

*From the Arizona State Laboratory, Tucson.
Received for publication, January 3, 1938.

Swabs of absorbent cotton are prepared in the usual manner. The untreated end of the wooden applicator is inserted into a cork stopper, and the swab is placed in a glass test tube, which the stopper fits snugly. The tubes are then sterilized by hot air, and after cooling, the swabs are immersed in sterile ascitic fluid under aseptic conditions, and are replaced in the test tubes. The tubes are then sealed (to prevent evaporation) by immersing the stopper and upper end of the tube in melted paraffin. The paraffin should be autoclaved before use, in order to prevent any possible contamination of the swabs by microorganisms which might be present in the paraffin. The ascitic fluid and the impregnated swabs must be tested for sterility by inoculation of a suitable culture medium. We prefer the "Difco" ascitic fluid for the preparation of these swabs. This material is collected from a large number of patients and is supplied in 10 c.c. ampoules which have been tested for sterility. This should eliminate any possible individual effects of inhibition of growth of the gonococcus. If ascitic fluid is secured from other sources, it may be preserved by the method suggested by Geiger:[4] "Permit the ascitic fluid to stand on ice for over night, and decant if necessary. Add 0.25 per cent chloroform and incubate 24 hours, shaking frequently. Test for sterility and preserve on ice as long as it is satisfactory and does not become too alkaline."

Smears are taken in the usual manner, and if necessary, a slide may be prepared immediately. We have found that, as a routine procedure, more satisfactory results are obtained by incubating the tubes overnight at 37° C. We have found that this period of incubation does not affect the appearance or staining properties of pus or epithelial cells and that if intracellular organisms are present, they may be readily observed. There is usually a marked increase in the number of extracellular organisms. A tentative diagnosis, thus obtained, may be confirmed by using the swab to inoculate plates of suitable culture media, and then proceeding with the cultural identification in the usual manner. These methods are discussed in the American Public Health Association Yearbook 1936-7 (pages 125-134), and by textbooks of laboratory methods.

We have noted that, in general, few gram-positive organisms are present in smears prepared from the swabs which have been incubated overnight. Usually, gram-negative diplococci are either present or absent. Further investigations are to be made, in order to determine whether ascitic fluid inhibits the growth of other organisms as well as the possible production of dissociated forms of other bacteria which might resemble the gonococcus in staining properties.

This method has given extremely satisfactory results in this laboratory, and we have been able to isolate and to definitely identify by cultural methods, the gonococcus from vaginal smears and from prostatic fluid in chronic cases when microscopic examinations gave no significant results.

This method has the advantage that the swab used for securing the material also serves as an enrichment medium for the growth of the organisms. The tubes may be incubated by carrying them in the pocket.

REFERENCES

1. Solé, A.: Schnellkultur von Diphtheriebazillen, Wien. klin. Wchnschr. **47**: 713, 1934.
2. Brahdy, M. B., Lenarsky, M., Smith, L. W., and Gaffney, C. A.: Rapid Method for Identification of Diphtheria Bacilli; Also New Method for Identification of Carriers of Diphtheria Bacilli, J. A. M. A. **104**: 1881, 1935.
3. Park, W. H., Williams, A. W., and Krumwiede, C.: Pathogenic Microorganisms, ed. 9, Philadelphia, 1929, Lea & Febiger, p. 373.
4. Geiger, J. C.: Bacteriology of Specific Communicable Diseases; Handbook of Public Health Bacteriology, San Francisco, 1937, J. W. Stacey, Inc., p. 41.

RELATION OF VITAMIN C TO THE SCHICK TEST IN THE GUINEA PIGS*

LYLE A. WEED, PH.D., AND ROBERTA FENLON, M.S., IOWA CITY, IOWA

FOR many years the vitamins have been considered as being directly or indirectly related to infectious processes. Since the chemical nature of ascorbic acid has been definitely determined, many investigators have tried to establish a definite relationship between certain specific infections and the concentration of the vitamin in the blood and tissues. Jungeblut[1] has shown that crystalline vitamin C will neutralize (or destroy) certain bacterial toxins, including that of diphtheria. This has been demonstrated to take place in vitro and in vivo.

If there is a relationship between the vitamin C level in the blood and tissues of an animal and the destruction of diphtheria toxin, there should be a similar relationship between ascorbic acid and the Schick reaction in individuals with low antitoxic immunity. That is to say, persons with a high vitamin C level should give negative Schick reactions, even though the antitoxic level is low or absent. We preferred to subject this idea to experiment, using guinea pigs in preference to patients because of convenience in manipulation and because all data could be obtained under more uniform laboratory conditions.

Twenty guinea pigs, with weights ranging between 250 and 300 gm., were kept for three weeks on the regular diet to obtain uniform vitamin levels. They were then put on a vitamin C-free diet of Sherman, LaMer, and Campbell.[2] Ten of the animals were given 10 mg. of crystalline ascorbic acid dissolved in freshly boiled distilled water and administered subcutaneously immediately. The total volume injected was 0.2 c.c. The remaining 10 animals served as controls and were given 0.2 c.c. distilled water subcutaneously. Vitamin C determinations on the blood plasma were done on each animal at the beginning of, and at intervals during the experiment, using the microtechnique of Farmer and Abt.[3] The final determinations were done on the day of, but prior to, the intracutaneous administration of varying amounts of diphtheria toxin. Each

*From the Department of Bacteriology, University of Iowa, Iowa City.
Received for publication, January 3, 1938.

animal was tested with 1, 2, 5, and 10 M. R. D. There was no difference in the amount or degree of the reactions among the two groups of pigs. There was a difference, however, in the rate at which they reacted; the ones without vitamin C required one to two days longer to produce the redness, swelling, edema, and necrosis.

We were very much disappointed to find that the vitamin C content of the two groups, as measured by the micromethod of Farmer and Abt for blood plasma, did not change during the experiment, despite the fact that some of the animals were in the well-developed stage of avitaminosis and others receiving 10 mg. daily were in good health.

In spite of this disappointment we repeated the experiment, giving graded doses of vitamin C, 0.5, 3, and 5 mg. daily. Again there was no difference in the reaction of the guinea pigs to intracutaneous injection of the toxin.

SUMMARY

1. There appeared to be no demonstrable difference in the degree of sensitivity to diphtheria toxin of guinea pigs receiving varying amounts of vitamin C and those not receiving it.

2. Guinea pigs showing scurvy did not react as rapidly to intracutaneous injections of diphtheria toxin as did the ones receiving vitamin C.

3. We were unable to apply the microtechnique of Farmer and Abt for vitamin C determination in blood plasma when guinea pigs were used as the experimental animal.

This work was done prior to the report of Pijoan and Klemperer using KCN. (J. Clin. Investigation **16**: 443, 1937.)

REFERENCES

1. Jungeblut, C. W., and Zwemer, R. L.: Inactivation of Diphtheria Toxin In Vivo and In Vitro by Crystalline Vitamin C (Ascorbic Acid), Proc. Soc. Exper. Biol. & Med. **32**: 1229, 1935.
2. Sherman, H. C., LaMer, V. K., and Campbell, H. L.: The Quantitative Determination of the Antiscorbutic Vitamin (Vitamin C), J. Am. Chem. Soc. **44**: 165, 1922.
3. Farmer, C. J., and Abt, A. F.: Determination of Reduced Ascorbic Acid in Small Amounts of Blood, Proc. Soc. Exper. Biol. & Med. **34**: 146, 1936.

A PRACTICAL METHOD OF STAINING TREPONEMA PALLIDA BY MEANS OF LOW SURFACE TENSION STAIN

ROBERT D. HAIRE, M.D., HOBBS, N. M.

MOST stains have as their solvent water, alcohol, or a combination of both.[1] Water has a surface tension of about 72 dynes per cm. and alcohol about 28 dynes per cm.[2] Millikan, Gale, and Coyle define surface tension as the tendency of any liquid to reduce its exposed surface to a minimum, that is, the tendency of any liquid surface to act like a stretched elastic skin or membrane. The penetrating power of any liquid is increased as the surface tension is lowered; this explains the penetrating power of hexylresorcinol solution, commonly

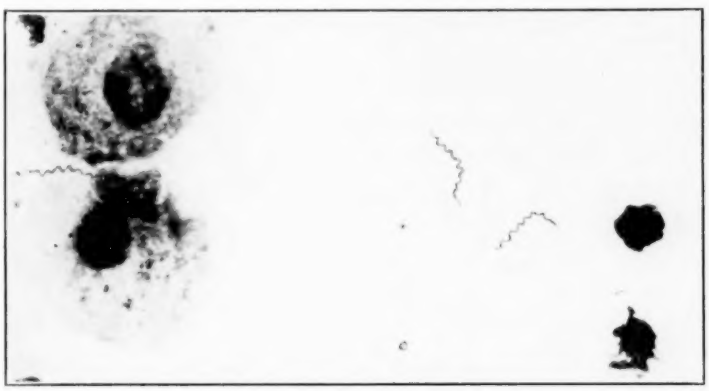


Fig. 1.

known as S. T. 37. Alcohol, though having a lower surface tension than hexylresorcinol, is a tissue coagulant which probably prevents its easily staining *Spirochaeta pallida*.

By making use of this low surface tension as a solvent and using gentian violet as the dye, it has been possible to successfully and easily stain the *Spirochaeta pallida* on smears obtained from primary lesions. Twelve cases of early primary syphilis were first checked and diagnosed by dark-field examination and, if found positive, stained in the method outlined below. All twelve cases took a clear stain and many typical spirochetes were found on all slides.

A stock solution of 1 per cent gentian violet stain is made by placing 1 gm. of powdered stain in a small mortar, and while mixing, slowly adding 100 c.c. of hexylresorcinol. This is then filtered and put in a stock solution bottle. It is ready for use and does not need refiltering.

Received for publication, March 31, 1938.

In obtaining material the primary lesion should be wiped clean with a towel until serum starts to appear, it being best not to have bleeding occur. Wait five minutes before obtaining smear, then make a thin smear on a clean glass slide, dry in air, and fix with heat. Apply stain for thirty minutes, wash in water, dry in air, and examine. The *Spirochaeta pallida* is found to stain a light purple on a faintly purple field and is easily recognized from its characteristics. Bacteria, pus cells, and epithelial cells also take a light purple stain.

As stated before the material was checked by use of dark-field examination. As an additional proof two sets of known *Spirochaeta pallida* slides obtained from primary lesions were furnished by Dr. C. Alexander Hellwig, of the Pathology Department, St. Francis Hospital, Wichita, Kansas. These were as easily stained as were the others obtained locally.

CONCLUSIONS

1. *Treponema pallida* is easily stained by making use of gentian violet with a low surface tension solvent.

2. There is no danger of overstaining, since slides were left in the staining jar for several hours with no deepening in intensity of stain, and were clear when examined.

3. The time of thirty-minutes has been given as a safe period for staining.

4. Under no condition must the stain be heated on the slide, as this will promptly remove the smear.

5. No apparent damage to the cement holding the lens of the oil immersion objective has been noted in eight months of use.

6. Apparently the surface tension of the solvent is changed very little by the addition of powdered gentian violet.

REFERENCES

1. Krall, G. H.: Medical Division, Sharp and Dohme. Answer to letter of inquiry, Feb. 24, 1938.
2. Millikan, Robert A., Gale, Gordon H., Coyle, James P.: New Elementary Physics, New York, 1936, Ginn & Co., p. 137.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

MENINGITIS, Meningococcus, Control of, Kuhns, D. M., Kisner, P., Williams, M. P., and Moorman, P. L. J. A. M. A. **110:** 484, 1938.

Value of Intradermal Skin Testing and Prophylactic Immunization.—Of 7,339 junior CCC enrollees in forty-eight different camps in Missouri, tested intradermally, 3,926, or 53.5 per cent, showed a one-plus or greater reaction; 3,517 of the original positive reactors were retested after an interval of approximately two months, and the reaction of 78.9 per cent had changed from positive to plus-minus or negative.

All enrollees with a one-plus or greater reaction were inoculated with 0.5, 1, 1.5, and 1.5 c.c. of full strength filtrate at four-day intervals. The greatest reactions were caused by the third dose. This dose produced mild systemic reactions, with temperatures occasionally at 101° or 102° F. No untoward reactions occurred.

One month after immunization, three enrollees in three different camps, with plus-minus reactions, who were not immunized, had meningococcic meningitis. After immunization of the remainder of the group, no further cases occurred.

Twenty-six cases of meningitis have occurred in CCC camps in Missouri in the past two years. None have occurred in those immunized persons in the same groups in the seven winter months since inoculation.

Results of the Use of Toxin in Twenty Epidemics and Beginning Outbreaks.—In some of the first outbreaks only persons with a one-plus or greater reaction were immunized. Later all persons were immunized, regardless of the reaction to the intradermal test.

Prior to giving the toxin there were 9 outbreaks of one case each. In the remainder of the outbreaks the number of cases varied from 2 to 12. Only one case has occurred in the period of from one month to one and one-half years since the immunizations were completed in the twenty camps.

In immunizing newly concentrated groups or groups in the presence of impending epidemics the authors believe that the safest procedure is not to depend on the intradermal test to differentiate the immune from the nonimmune, but to inoculate the entire group.

Further trial of the full strength meningococcus filtrate as an active immunizing agent against epidemics of meningitis is warranted.

BLOOD: The Leukocyte Picture in Iraq, Kennedy, W. P. Tr. Roy. Soc. Trop. Med. & Hyg. **31:** 309, 1937.

The differential leucocyte count and polynuclear index of 400 carefully controlled healthy persons in Iraq (the majority being British airmen) are described and statistical tests have been applied to the data. The statistics show the variation of the population is consistent with the normal curve of frequency distribution, as would be expected on general physiologic grounds.

On comparing the results with an estimate derived from standard textbooks of the differential blood picture in temperate climates, it is found that in Iraq the percentage of neutrophiles is decreased, the percentage of eosinophiles, lymphocytes, and monocytes is increased. The polynuclear index is significantly lowered or deviated to the left.

The range of normal variation is much wider than is given in any of the available textbooks.

The incidence of so-called abnormal cells, or cells not usually present in normal blood, is found to be greater than reported in standard works. This is taken to be evidence of irritability or greater activity of the hemopoietic system, and the contention is supported by the deviation of the polynuclear index.

It is suggested that the cause of the alterations in the blood are climatic, but the detailed discussion of the evidence for this is left to a future paper.

No other generally acting factor could be found, to which the blood changes could be ascribed, though the possibility of such a factor's existing is recognized.

Similar studies giving statistics and comparative standards are presented on the blood in malaria, tuberculosis, leprosy, phlebotomus fever, and bilharziasis. As would be expected in all these diseases, the blood picture differs significantly from the control group. The polynuclear index shows a shift to the left as compared with the normals, and it is concluded that the value of this test is not invalidated by the deviation of the control group from British standards, for under pathologic stimuli it becomes a fortiori greater.

It is concluded that in Iraq, at least, monocytosis is not a diagnostic characteristic of malaria.

Considerable variation is found in the differential count of the tubercular cases, and the impression is gained that the ranges are greater than those in similar cases in Britain. The polynuclear count is also significantly deviated to the left as compared with a British group of tubercular patients.

Relative lymphocytosis is a characteristic of the blood of lepers in Iraq. It is remarked that basophiles are commoner in lepers than in the other groups.

The leucocytic alterations in phlebotomus fever are profound and attention is drawn particularly to the very low polynuclear index, and to the prolonged after-effects of the disease.

The wide range of eosinophiles in bilharziasis is noted. The polynuclear count is just significantly deviated.

PURPURA, Suction Test for Capillary Resistance in Thrombocytopenic, Elliott, R. H. E. J. A. M. A. **110:** 1177, 1938.

The suction method of measuring capillary resistance is a simple, relatively accurate means of following the frequent fluctuations in the permeability of the capillaries in patients with thrombocytopenic purpura.

The capillary resistance in thrombocytopenic purpura tends to parallel the bleeding tendency rather than the platelet level.

The suction method of measuring capillary resistance is a useful means of estimating the activity of the disease process in patients with thrombocytopenic purpura and may, in certain instances, be of more value in this connection than the platelet count.

In seven cases of idiopathic thrombocytopenic purpura, the capillary resistance rose abruptly during the first twenty-four hours after removal of the spleen, and in one instance doubled the preoperative level before closure of the abdominal wound had been completed.

After splenectomy for idiopathic thrombocytopenic purpura, the capillary resistance may be the first measurable entity to be materially affected, the rise in resistance preceding that of the platelets.

MENINGITIS, Meningococcic and Acute Meningococcemia, Craster, V. C., and Simon, H. J. A. M. A. **110:** 1070, 1938.

A thorough knowledge of the bacteriology, anatomy, and physiology of the meninges and the cerebrospinal fluid is essential for the proper early diagnosis and treatment of meningococcic infections.

Meningococcic infections have a low incidence and a high mortality. There were 572 cases in the city of Newark during the past nineteen years, with a mortality of 44.4 per cent.

A petechial or purpuric rash may be considered the specific rash of meningococcemia. A purpuric rash accompanies the petechial rash when the condition is fulminating. The rash occurs before the spinal fluid becomes cloudy and before the onset of meningeal signs, and persists after definite meningeal signs appear. In many cases meningococci can be demonstrated by a Gram stain of blood obtained by needle puncture of a petechial or purpuric area. A Wright stain of blood from a nonpurpuric skin may also demonstrate meningococci.

Acute fulminating meningococcemia associated with adrenal hemorrhages has been termed the Waterhouse-Friderichsen syndrome.

A meningococcic infection attacked four children in one family, with recovery of two and death of the other two, and post-mortem examination revealed the typical pathologic changes in the Waterhouse-Friderichsen syndrome. Cases 3 and 4 of this group illustrate the quick death of the patient, within twenty-four to thirty-six hours after the onset of symptoms. Negative tests of the spinal fluid have been reported in a majority of similar cases.

Three of the cases reported illustrate toxic encephalitis secondary to meningococcic meningitis; in such cases striking deviations from the normal mentality attract the attention of the experienced worker.

Two patients had initial complaints of pain and tenderness in one joint, necessitating a tentative diagnosis of monoarticular arthritis. The stiff neck and Kernig sign did not appear until two days after the onset of arthritic symptoms.

In infancy, particularly in the first and second years of life, the early signs of meningococcic infection of the meninges do not point to involvement of the central nervous system. They are more likely to suggest an infection of the upper respiratory tract or a gastrointestinal disturbance. After the disease is well established, the stiff neck, the Kernig and Brudzinski signs, and other meningeal signs appear.

RHEUMATIC FEVER, Cytologic Studies of, McEwen, C. Arch. Path. **25**: 303, 1938.

A comparative study is reported of supravitally stained preparations of subcutaneous nodules from patients with rheumatic fever, rheumatoid arthritis, and syphilis. The cells of rheumatoid arthritic nodules were found to have essentially the same characteristics as those of rheumatic fever. The cells of syphilitic nodules differed and proved to be those characteristic of syphilitic lesions in general. The bearing of these results on the nature of the characteristic cells of rheumatic granulomas in the heart and on the relationship between rheumatic fever and rheumatoid arthritis is discussed.

TRICHINOSIS, Epidemic of Sixty-Four Cases, Ferenbaugh, L. T., Segal, L., and Schulze, H. A. J. A. M. A. **110**: 1434, 1938.

In this epidemic there were 44 patients with trichinosis who required hospital treatment and 20 ambulatory patients with little or no sick record.

An average incubation period of twelve and four-tenths days was definitely established for the epidemic.

Five quantitative biopsies showed from 8 to 800 living larvae per gram of muscle.

A preliminary survey of work now being done on autopsy material by the National Institute of Health, United States Public Health Service, indicates that many individuals have become infested with trichina larvae.

The improved antigen demonstrated a dependable accuracy in the cutaneous tests of 180 persons concerned in the epidemic and 50 control subjects.

Preventive measures against trichinosis should include prevention of the disease in swine as well as in man.

BLOOD TRANSFUSION, Renal Insufficiency From, DeGowen, E. L., Warner, E. D., and Randall, W. L. Arch. Int. Med. **61**: 609, 1938.

The transfusion of canine hemoglobin into dogs when the urine is acid results in death from renal insufficiency. This does not occur when the urine is alkaline at the time of the transfusion.

The anatomic picture of obstruction of the renal tubules by hemoglobin pigment sufficient to be the chief cause of the renal insufficiency is observed in most dogs under the experimental conditions outlined. A nephrotoxic process often operates and may cause renal insufficiency independently.

The deposition of hemoglobin pigment as hemosiderin in the renal tubules and in the reticulo-endothelial system apparently does not contribute to the development of renal insufficiency.

An anatomic study of the kidneys of nine human beings who died of renal insufficiency after hemolysis revealed the two independent mechanisms seen in dogs, the obstruction with pigment and the necrosing factor.

In occasional human beings the precipitation of hemoglobin pigment in the tubules is extensive and may be a cause of renal insufficiency. This complication could probably be prevented by alkalinizing the urine prior to the transfusion.

The renal insufficiency after hemolysis in the majority of human beings is probably caused by some nephrotoxic substance which causes degeneration of tubular epithelium and interstitial edema.

AMEBIASIS, Complement Fixation in, Paulson, M., and Andrews, J. Arch. Int. Med. **61**: 562, 1938.

Serums from 150 persons studied clinically and parasitologically were submitted for complement fixation tests to one or more of three different laboratories in which one or more of three different types of specific antigens were employed.

When the patients were assembled into roughly homogeneous clinical groups, the incidence of positive results of complement fixation was regularly higher for those in whom *E. histolytica* was found microscopically than for those in whom the organism was not found. For the 150 patients considered as a whole the difference was statistically significant. For the 136 patients with symptoms it was even more highly significant, but in none of the clinical subdivisions was it statistically valid. Thus the results confirm in general the specificity of the technique developed by Craig and others. However, from a practical diagnostic standpoint the too numerous falsely positive as well as falsely negative responses obtained under the conditions of observation show that the test is unreliable in the individual case.

This reaction is not helpful in the cases in which it might be most useful to the clinician, in that the least significant coincidences occur in the cases of so-called indeterminate diarrhea and dysentery. In cases of chronic ulcerative colitis and chronic or intermittent diarrhea many positive reactions are obtained that are unassociated with demonstrable amebiasis.

While the general mass of serologic reports from different laboratories and with different antigens showed a fair degree of agreement, conspicuous disagreement occurred in some instances.

Of the various antigens and serologic systems employed, the most successful results, based on parasitologic diagnoses, were obtained with the Arnold antigen in Arnold's laboratory.

Statistical analysis of the partially positive reactions indicated the desirability of including them as positive reactions when obtained with relatively weak antigens and of excluding them from consideration when obtained with very sensitive antigens.

The authors are of the opinion that complement fixation is at present a diagnostic aid of adjunctive rather than of primary value. Its more successful application in clinical practice awaits further refinement. It should not be relied on as a diagnostic criterion unsupported by parasitologic evidence of infection. Notwithstanding the many defects of microscopic fecal diagnosis of amebiasis, this method is today more dependable than complement fixation.

BLOOD STAINS AND SEMINAL STAINS, Typing of, by Means of the Absorption Test, Landé, K. E. Arch. Path. 25: 464, 1938.

The fallacies and objections to the absorption test may be thus summarized:

1. The group qualities are not individual characteristics. Therefore, we cannot state that a certain blood stain definitely came from a certain victim but only that it might have come from such a source.

2. The examination is obviously without value if the suspect claims that the stain in question comes from himself and if he is of the same group as the victim.

3. In view of the progressive deterioration of agglutinins it is for the time being not always possible to recognize positively group O.

4. The factors which destroy the group substances are not understood sufficiently. The absence of the group substances cannot be interpreted as proof that a certain stain has not come from an individual either A or B. For the same reason the presence of A or B substance may not exclude with absolute certainty the possibility of group AB. At the present time all that can be stated is that factor A or B has been found, but not that the blood stain belongs to group A or B.

5. Group-specific differentiation is not limited to blood. Group substances are found in secretions and excretions of the body. They are present in saliva, sweat, and urine, often in greater concentration than in the blood. If one considers the ease and frequency with which clothing, linen, and similar articles are polluted with the aforementioned substances, it can readily be seen how the determination of the group of the blood stains on such articles may be frustrated.

6. One must also consider the group-specific differentiation of material of animal origin and in this connection the similarity of group substances of animals to those of human beings.

7. Finally, the effects of sunlight, chemicals, dyes, etc., on agglutinogens are insufficiently understood. The uncertain effects of these factors do not permit at the present time any extensive application of the determination of blood stains in the field of criminology.

The absorption test was carried out on 109 dried blood specimens and 93 stains obtained from seminal material, which came from subjects whose blood groups were originally known. In 94.5 per cent of the cases it was possible to determine correctly the group of the blood stains, and in 93.6 per cent of the cases this was possible with the seminal stains.

Of the 109 initial tests on blood, 14 (13 per cent), and of the 93 initial tests on seminal stains, 11 (12 per cent), yielded incorrect results. By repetition these mistakes could be brought down to 5.5 per cent.

SULFANILAMIDE, Studies of the Mode of Action of, Osgood, E. E., and Brownlee, I. E. J. A. M. A. 110: 349, 1938.

The major action of sulfanilamide on the beta-hemolytic streptococcus seems to be neutralization of the toxins. Either because of this action or incidentally it also decreases the rate of cell division of this organism. It appears not to kill these organisms directly, although it does permit the bactericidal properties of human serum and to some extent phagocytosis by leucocytes to kill organisms which they otherwise would be unable to kill. It has no direct effect on phagocytosis.

The effective concentration of sulfanilamide would appear to be about 1:100,000, or only one-tenth of that now ordinarily maintained in the blood stream, but this experimental observation requires confirmation by carefully controlled experiments on large numbers of human infections before it is justifiable to employ smaller dosages in dangerously ill patients. Sulfanilamide in concentrations even greater than those generally employed clinically does not appear to have direct toxic action on the nucleated cells of the majority of bloods or marrows. This does not exclude the occurrence of an occasional idiosyncrasy in the reactions of these cells such as is known to occur for other benzene ring drugs.

The possible effectiveness of and the mode of action of sulfanilamide on all other organisms known to produce human disease should be determined by the methods described as soon as possible. Cultures of human marrow should aid materially in the study of the mode of action of both noxious and therapeutic agents.

TISSUES: Clearing and Staining of Embryos for Demonstrating Ossification, Richmond, G. W., and Bennett, L. Stain Technol. 13: 77, 1938.

1. The fresh embryo or fetus is eviscerated through a small midline abdominal incision. This permits free access of the reagents, and prevents the liver pigments from staining the final mounting solution. The brain is not removed. The specimen is fixed in 95 per cent alcohol for two weeks or more. The longer it is fixed, the less likely it is to macerate in hydroxide. Formalin-fixed specimens may be cleared by varying the procedure as indicated.

2. Rinse the specimen in tap water and place in 1 per cent potassium carbonate for four weeks or longer.

3. Clear the soft parts by placing the specimen in 1 per cent potassium hydroxide for ten days or longer, until the bones are plainly visible through the soft tissues. Formalin-fixed embryos will require 10 per cent potassium hydroxide for about a month. If the tissues become too soft in the clearing solution, the appendages may break away. This is remedied by hardening the preparation in a solution of equal parts of glycerin, 95 per cent alcohol, and water for twelve to twenty-four hours, after which the specimen may be returned to the clearing solution with safety. The potassium hydroxide may be reduced to 0.5 per cent the last few days of clearing.

4. Wash in running tap water for twelve hours.

5. Stain for thirty to sixty minutes by submerging the specimen in a freshly prepared 0.1 per cent aqueous solution of alizarin red S (alizarin monosodium sulfonate) to which 6-10 drops of 1 per cent potassium hydroxide are added.

Item

The American College of Physicians

The Twenty-Third Annual Meeting of the American College of Physicians will be held in New Orleans, with general headquarters at the Municipal Auditorium, March 27-31, 1939.

Dr. William J. Kerr of San Francisco, president of the College, will have charge of the program of general scientific sessions. Dr. John H. Musser of New Orleans has been appointed general chairman of the session, and will be in charge of the program of clinics and demonstrations in the hospitals and medical schools and of the program of round-table discussions to be conducted at the headquarters.